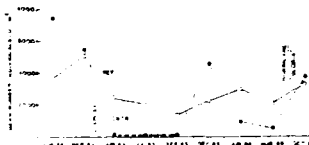
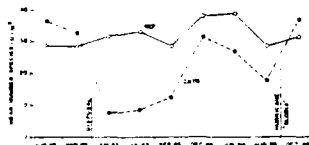




US Environmental
Protection
Agency

AD-A200 175

File
AD-A200 175



US Army Corps
of Engineers

FILE COPY

FIELD VERIFICATION PROGRAM
(AQUATIC DISPOSAL)

2

TECHNICAL REPORT D-88-5

SYNTHESIS OF RESEARCH RESULTS: APPLICABILITY AND FIELD VERIFICATION OF PREDICTIVE METHODOLOGIES FOR AQUATIC DREDGED MATERIAL DISPOSAL

by

J. H. Gentile, G. G. Pesch, J. Lake, P. P. Yevich
G. Zaroogian, P. Rogerson, J. Paul, W. Galloway
K. Scott, W. Nelson, D. Johns, W. Munns

Environmental Research Laboratory
US Environmental Protection Agency
Narragansett, Rhode Island 02882

DTIC
ELECTE
NOV 08 1988
S D



September 1988

Final Report

Approved For Public Release; Distribution Unlimited

88 11 08 020

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and

US Environmental Protection Agency
Washington, DC 20460

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
PO Box 631, Vicksburg, Mississippi 39181-0631

Destroy this report when no longer needed. Do not return
it to the originator.

The findings in this report are not to be construed as an official
Department of the Army position unless so designated
by other authorized documents.

The contents of this report are not to be used for
advertising, publication, or promotional purposes.
Citation of trade names does not constitute an
official endorsement or approval of the use of
such commercial products.

The D-series of reports includes publications of the
Environmental Effects of Dredging Programs:
Dredging Operations Technical Support
Long-Term Effects of Dredging Operations
Interagency Field Verification of Methodologies for
Evaluating Dredged Material Disposal Alternatives
(Field Verification Program)

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited.		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE			5. MONITORING ORGANIZATION REPORT NUMBER(S) Technical Report D-88-5		
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			7a. NAME OF MONITORING ORGANIZATION USAEWES Environmental Laboratory		
6a. NAME OF PERFORMING ORGANIZATION USEPA, Environmental Research Laboratory		6b. OFFICE SYMBOL (if applicable)	7b. ADDRESS (City, State, and ZIP Code) PO Box 631 Vicksburg, MS 39180-0631		
6c. ADDRESS (City, State, and ZIP Code) Narragansett, RI 02882		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER			
8a. NAME OF FUNDING / SPONSORING ORGANIZATION See reverse		8b. OFFICE SYMBOL (if applicable)	10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State, and ZIP Code) Washington, DC 20314-1000; Washington, DC 20460		PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Synthesis of Research Results: Applicability and Field Verification of Predictive Methodologies for Aquatic Dredged Material Disposal					
12. PERSONAL AUTHOR(S) See reverse					
13a. TYPE OF REPORT Final report		13b. TIME COVERED FROM _____ TO _____		14. DATE OF REPORT (Year, Month, Day) September 1988	
				15. PAGE COUNT 76	
16. SUPPLEMENTARY NOTATION Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Aquatic disposal; Dredged material; Biological effects; Field Verification Program; Black Rock Harbor; Marine pollution; (K7)		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The Field Verification Program was designed to determine the applicability, reproducibility, and field verification of test methods for the evaluation of disposal of dredged material at aquatic, upland, and wetland sites. There were three objectives in this program. The first was to demonstrate the applicability of existing test methods to detect and measure effects of dredged material and to determine the degree of variability and reproducibility inherent in the testing procedures. The second objective was to field verify the laboratory responses by comparing the exposure-response relationships between the laboratory and field. The third objective was to determine the degree of correlation between contaminated tissue residues and biological responses resulting from laboratory and field exposure to dredged material. These objectives were examined for the following biological responses: bioaccumulation, scope for growth, bioenergetics, adenylate energy charge, sister chromatid exchange, (Continued)					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

8a. FUNDING/SPONSORING ORGANIZATION (Continued).

US Army Corps of Engineers;
US Environmental Protection Agency

12. PERSONAL AUTHORS (Continued).

Gentile, J. H.; Pesch, G. G.; Lake, J.; Yevich, P. P.; Zaroogian, G.; Rogerson, P.; Paul, J.; Galloway, W.; Scott, K.; Nelson, W.; Johns, D.; Munns, W.

19. ABSTRACT (Continued).

histopathology, survival, growth, reproduction, intrinsic rates of population growth, recolonization, and community structure.

Survival in 11 aquatic species representing four phyla was determined for exposure to Black Rock Harbor (BRH) bedded and suspended sediments as part of initial sediment characterization studies. Only *Ampelisca abdita* showed acute mortality from bedded sediment exposures. Burrowing activity was impaired in the polychaete *Nephtys incisa* and the mollusc *Yoldia limatula*, and tube building was impaired in the infaunal crustacean *A. abdita*. No acute effects were noted with epibenthic or water column species exposed to either the solid phase alone or in combination with the suspended sediment phase exposures of 25 mg BRH sediment per litre.

The laboratory documentation studies demonstrated that the biological tests examined in the program could be applied to measuring the effects of dredged material on marine species in the laboratory with reproducible results. However, of the responses studied, adenylate energy charge and sister chromatid exchange did not respond predictably, quantitatively, or reproducibly to graded exposures of dredged material. It must be pointed out that the histopathological responses were qualitatively reproducible in that similar lesions occurred in the same species repeatedly, although the frequency of the response was variable.

The field verification component was designed to verify the responses of the laboratory test methods in the field. The operative null hypothesis states that exposure-response relationships developed in the laboratory are not significantly different from those in the field. Since the acquisition of real-time, continuous, field exposure data was beyond the scope of this project, exposure boundaries were estimated for the near-bottom water column, sediment/water interface, and infaunal compartments. Bioaccumulation, scope for growth, bioenergetics, adenylate energy charge, sister chromatid exchange, and histopathology test methods were applied to the same species in both the laboratory and field. However, the correspondence between estimated laboratory and field responses to dredged material varied with the type of response and the species tested.

There was concurrence between laboratory and field bioaccumulation of polychlorinated biphenyl and polycyclic aromatic hydrocarbon contaminant classes, chemical patterns, and the magnitude of accumulation in both *M. edulis* and *N. incisa*. Likewise, measures of scope for growth and shell growth in *M. edulis* and of respiration, excretion, and histopathology in *N. incisa* corresponded between the laboratory and field when analogous exposure conditions were compared. Adenylate energy charge and sister chromatid exchange measured in both the laboratory and the field did not respond to graded exposures of BRH dredged material. The results of these studies indicate that certain laboratory test methods, which respond in an exposure-related manner to dredged material, can be field verified when analogous exposures are present in the laboratory and field.

The following recommendations are made regarding the application of the test methods in this study for dredged material evaluation: (a) measures of survival, growth, reproduction, population, scope for growth, and contaminant bioaccumulation are recommended in the predisposal evaluation of dredged material; (b) while a genotoxic test method is desirable in the predisposal evaluation, sister chromatid exchange is not recommended pending further development of the test method; (c) acceptable methods for postdisposal field assessments include scope for growth, growth, and bioaccumulation measured in *M. edulis* and bioenergetics and bioaccumulation in *N. incisa*; (d) benthic community assessment methods, such as REMOTS, are recommended for rapid reconnaissance applications and species classification and enumeration for definitive assessments.

The conclusions from this study demonstrate the ability to apply a variety of test methods to the evaluation of dredged material impacts, to verify these methods under field conditions, and to recommend methods for the predisposal evaluation and postdisposal monitoring of dredged material impacts.

PREFACE

This report describes work performed by the US Environmental Protection Agency (USEPA), Environmental Research Laboratory, Narragansett, R. I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). The FVP was sponsored by the Office, Chief of Engineers (OCE), US Army, and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing predictive techniques for evaluating the environmental consequences of dredged material disposal under aquatic, intertidal, and upland conditions. The aquatic portion of the FVP was conducted by ERLN, with the wetland and upland portions conducted by WES.

Technical support was provided by Meses. Carol Pesch, Suzanne Lussier, and Carolyn Yevich, Drs. A. Russell Malcolm, John Paul, Gerald Hoffman, and Donald Phelps, and Mr. Steven Schimmel of ERLN, and by Meses. Cornelia Mueller, Ruth Gutjahr-Gobell, and Michelle Redmond and Drs. Donald Rhodes and Richard Pruell of Science Application International Corporation. The authors extend thanks to the following persons: Dr. Anthony Calabrese, Director, and Mr. Robert Alix, Captain of the *Shang-Wheeler*, at the National Oceanic and Atmospheric Administration Milford Laboratory for their support throughout the program; Mr. Jeffrey S. Rosen of Computer Sciences Corporation (CSC) for the development and operation of the data management system used in this research program; and Ms. Joan E. Seites, CSC, for manuscript preparation and word processing support.

The USEPA Technical Director for the FVP was Dr. John H. Gentile; the Technical Coordinators were Dr. Gerald Pesch and Mr. Walter R. Galloway; and the Program Managers were Mr. Allan D. Beck and Dr. Richard L. Latimer.

The study was conducted under the direct WES management of Drs. Thomas M. Dillon, Richard K. Peddicord, and Thomas D. Wright and under the general management of Dr. C. Richard Lee, Chief, Contaminant Mobility and Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison, Chief, Environmental Laboratory. The Environmental Effects of Dredging Programs Manager was Dr. Robert M. Engler, with Mr. Robert L. Lazor, FVP Coordinator. This report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.



Dist	Area Director Special
A-1	

Codes

The OCE Technical Monitors were Drs. John Hall, Robert J. Pierce, and William L. Klesch. The Water Resources Support Center Technical Monitor was Mr. Charles W. Hummer.

COL Dwayne G. Lee, CE, was Commander and Director of WES. Dr. Robert W. Whalin was Technical Director.

This report should be cited as follows:

Gentile, J. H., et al. 1988. "Synthesis of Research Results: Applicability and Field Verification of Predictive Methodologies for Aquatic Dredged Material Disposal," Technical Report D-88-5, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

CONTENTS

	<u>Page</u>
PREFACE.....	1
LIST OF TABLES.....	4
LIST OF FIGURES.....	4
PART I: INTRODUCTION.....	5
Background.....	5
Project Description.....	7
Project Scope.....	9
Laboratory-to-Field Comparisons.....	10
Residue-Effects Comparisons.....	11
Laboratory and Field Experimental Designs.....	11
Laboratory Exposure.....	12
Field Exposure.....	14
PART II: BIOLOGICAL RESPONSES IN THE LABORATORY AND FIELD.....	18
Scope for Growth.....	18
Bioenergetics.....	21
Adenylate Energy Charge.....	24
Sister Chromatid Exchange.....	31
Histopathology.....	32
Bioaccumulation.....	38
Population Response Parameters.....	42
Benthic Recolonization and Community Structure.....	45
PART III: DISCUSSION.....	52
Species and Response Sensitivity.....	52
Biological Response Summary and Evaluation.....	53
Field Verification.....	57
Residue-Effects Relationships.....	59
PART IV: CONCLUSIONS.....	61
Conclusions.....	61
Recommendations.....	66
REFERENCES.....	68

LIST OF TABLES

<u>No.</u>		<u>Page</u>
1	Concentrations of the 10 Selected Contaminants and Two Summary Statistics for BRH and Reference Sediments.....	10
2	Marine Species and Biological Responses Studied in the FVP.....	13
3	Range of Field Exposures at the Disposal Site.....	16
4	Comparative Acute and Chronic Toxicity of Reduced and Oxidized BRH Sediment to <i>M. bahia</i> and <i>A. abdita</i>	43
5	Ranking of Response Sensitivity in <i>M. edulis</i> and <i>N. incisa</i> to BRH Sediment Exposure.....	53
6	Success and Applicability of Test Methods for Evaluating Dredged Material Effects.....	62

LIST OF FIGURES

<u>No.</u>		<u>Page</u>
1	Central Long Island Sound disposal site and Black Rock Harbor dredging site.....	7
2	FVP disposal site and station locations.....	8
3	Exposure-response relationship for SFG in <i>M. edulis</i> measured in the laboratory and field.....	21
4	Exposure-response relationship for ammonia excretion rates in <i>N. incisa</i> measured in the laboratory and field.....	23
5	Exposure-response relationship for respiration rate in <i>N. incisa</i> measured in the laboratory and field.....	24
6	Exposure-response relationship for AEC in <i>M. edulis</i> in the laboratory and field.....	26
7	Exposure-response relationship for total adenine pool in <i>M. edulis</i> in the laboratory and field.....	27
8	Change in AEC in <i>N. incisa</i> exposed to BRH suspended sediment in the laboratory and field.....	29
9	Percent change in adenate nucleotides in <i>N. incisa</i> exposed to BRH suspended sediments in the laboratory and field.....	30
10	Percent incidence of gill pathology in <i>M. edulis</i> measured in the laboratory and field.....	34
11	Percent incidence of gastrointestinal tract pathology in <i>M. edulis</i> measured in the laboratory and field.....	35
12	Exposure-response relationship for epidermal thickening in <i>N. incisa</i> in the laboratory and field.....	37
13	Field verification of tissue residues (PCB) in <i>M. edulis</i>	40
14	Field verification of tissue residues (PCB) in <i>N. incisa</i>	41
15	Temporal and spatial patterns of the community parameters, successional stage, and organism sediment index at the FVP disposal site.....	49
16	Temporal and spatial patterns of the mean number of species per quadrat and mean density of all individual infaunal organisms at the FVP disposal site.....	50

SYNTHESIS OF RESEARCH RESULTS: APPLICABILITY AND FIELD VERIFICATION
OF PREDICTIVE METHODOLOGIES FOR AQUATIC DREDGED MATERIAL DISPOSAL

PART I: INTRODUCTION

Background

1. The Marine Protection, Research, and Sanctuaries Act (Public Law 92-532) was passed by Congress in 1972. This law states that it is the policy of the United States to regulate disposal of all types of materials into ocean waters and to prevent or strictly limit disposal of any material that would adversely affect human health or welfare, the marine environment, or ecological systems. The implementation of this law, through the issuance of permits as defined in the final regulations and criteria, is shared jointly by the US Environmental Protection Agency (USEPA) and the US Army Corps of Engineers (CE).

2. In 1977, the CE and the USEPA prepared technical guidance for implementation of the final ocean dumping regulations in the form of a manual entitled "The Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters" (USEPA/CE 1977). This manual specified which test procedures were to be followed in collecting information to be used in making a disposal decision. Among the procedures were those for: (a) chemically characterizing the proposed dredged material; (b) determining the acute toxicity of liquid, suspended particulate, and solid phases; (c) estimating the potential contaminant bioaccumulation; and (d) describing the initial mixing during disposal. These methods have been used for determining the suitability of dredged material for open-water disposal. The procedures in this manual represented the technical state of the art at that time and were never intended to be inflexible methodologies. The recommended test methods were chosen to provide technical information that was consistent with the criteria specified in the regulations. However, use of the manual in the permit process has identified both conceptual and technical limitations with the currently recommended test methods (Gentile and Scott 1986).

3. To address the limitations of the testing program, the Interagency Field Verification of Testing and Predictive Methodologies for Dredged

Material Disposal Alternatives Program or the Field Verification Program (FVP) was authorized in 1982. This 6-year program was sponsored by the Office, Chief of Engineers, and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing test methods for predicting the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP was conducted by the USEPA Environmental Research Laboratory, Narragansett, R. I. (ERLN). The wetland and upland portions, conducted by WES, are reported in separate documentation.

4. The USEPA ERLN was responsible for conducting research on the aquatic disposal of dredged material. There were three research objectives for this portion of the program. The first was to demonstrate the applicability of existing test methods to detect and measure effects of dredged material, and to determine the degree of variability and reproducibility inherent in the testing procedure. This phase of the program (Laboratory Documentation) was published in a series of technical reports that provide insight into how the various methods function, their sources of variability, their respective and relative sensitivities to the specific dredged material being tested, and the degree of confidence that can be placed in the data derived from the application of the methods.

5. The second objective was to field verify the laboratory responses by measuring the same response under both laboratory and field exposures. A basic and often implicit assumption is that results derived from laboratory test methods are directly applicable in the field. While this assumption is intuitive, there are no supporting data from studies on complex wastes in the marine environment. The study reported herein offers a unique opportunity to test this basic assumption.

6. The third objective was to determine the degree of correlation of tissue residues resulting from bioaccumulation of dredged material contaminants with biological responses from laboratory and field exposure to dredged material. However, this study was not designed to address cause-effect relationships, and the multicontaminant nature of the dredged material precludes any such assumptions.

Project Description

7. The disposal site for the FVP was a historical site known as the Central Long Island Sound (CLIS) disposal site (1.8 by 3.7 km), located approximately 15 km southeast of New Haven, Conn. (Figure 1). The

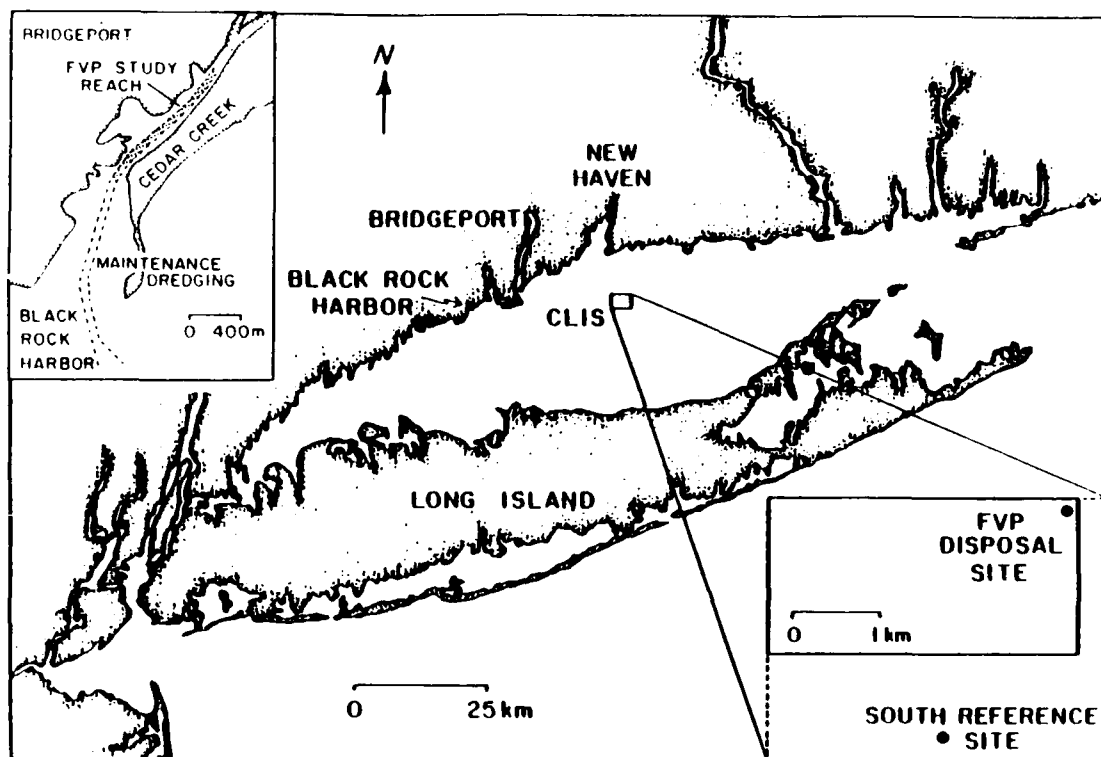


Figure 1. Central Long Island Sound disposal site and Black Rock Harbor dredging site

sedimentology at the disposal and reference sites is primarily silt-clay, with a mean grain size of 0.013 mm. Thermal stratification occurs from April to September, and during this period bottom salinity is slightly higher than that of the surface. Tidal currents typically dominate the near-bottom water in an east-west direction. Suspended sediment concentrations average 10 mg/l, with storm-induced values approaching 30 mg/l, measured 1 m above the bottom. The baseline biological community data revealed a homogeneous, mature infaunal community dominated by the polychaete *Nephtys incisa* and the bivalve molluscs *Mucula proxima* and *Yoldia limatula*.

8. The FVP disposal site was selected within the CLIS so as to minimize contamination from other sources, including relic disposal operations and ongoing disposal activities occurring during the study period. This was necessary to ensure a point source of contamination for the study. The uniformity of physical, chemical, and biological properties of the disposal site prior to disposal allowed detection of changes in these properties due to the disposal of the dredged material. Finally, the stations used to monitor the biological effects in this study were selected along the primary axis of tidal current flow to represent a gradient of potential exposure for the biota (Figure 2).

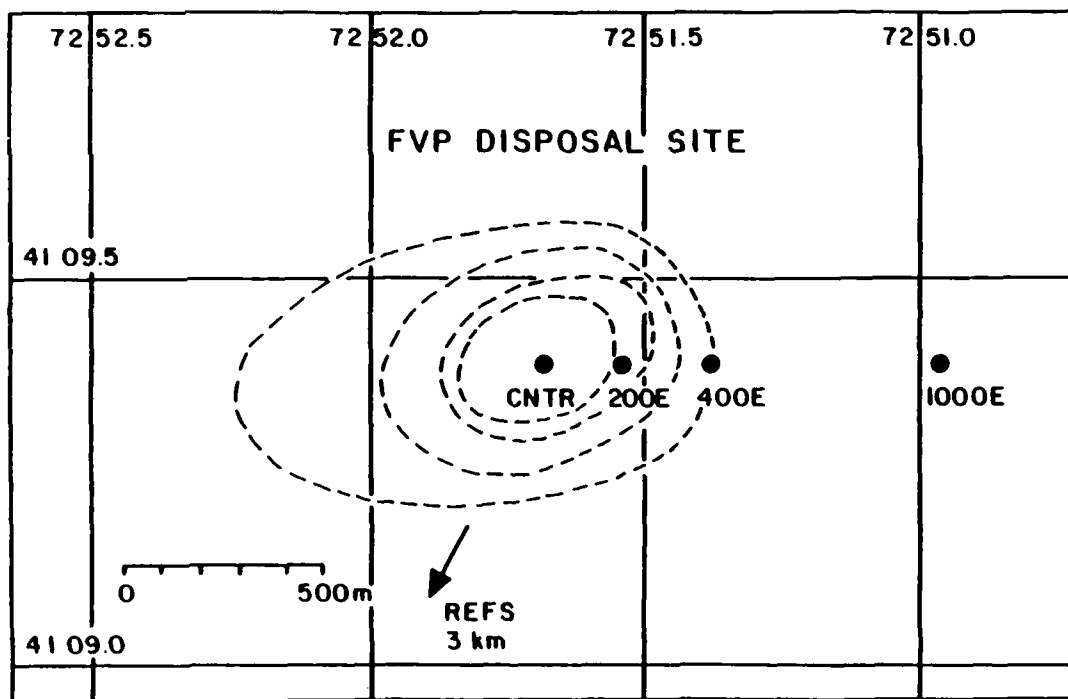


Figure 2. FVP disposal site and station locations

9. The spatial scale of this study was near-field and limited to the immediate vicinity of the disposal site. A primary assumption was that the mound of dredged material constituted a point source of contamination. The temporal scale for the study was 4 years, which included a year of predisposal data collection to define seasonal patterns in the physical, chemical, and biological variables and 3 years of postdisposal data collection to address

the objectives of the program and to evaluate the long-term impacts of the disposal operation on the surrounding benthic communities.

10. The dredging site was Black Rock Harbor (BRH), located in Bridgeport, Conn., where maintenance dredging provided a channel 46 m wide and 5.2 m deep at mean low water (Figure 1). Approximately 55,000 m³ of material was dredged during April and May 1983 and disposed in 20 m of water in the north-eastern corner of the CLIS disposal site.

11. The dredged material from BRH contained substantial concentrations of both organic and inorganic contaminants. A subset of the total contaminant profile examined in this project is presented as Table 1 (Pesch et al. 1987). Polychlorinated biphenyls (PCBs) were present in the dredged material at a concentration of 6,400 ng/g, and polynuclear aromatic hydrocarbons (PAHs) with molecular weights between 166 and 302 were present at concentrations ranging from 1,000 to 12,000 ng/g. Alkyl homologs of the PAHs were also present in the dredged material at concentrations between 1,000 and 13,000 ng/g. Inorganic contaminants of toxicological importance present in the dredged material included copper (2,900 µg/g), chromium (1,480 µg/g), zinc (1,200 µg/g), lead (380 µg/g), nickel (140 µg/g), cadmium (24 µg/g), and mercury (1.7 µg/g).

Project Scope

12. The FVP was unique among marine research studies for several reasons. The program objectives were directly focused on addressing specific limitations in the methods and interpretive framework of the current regulatory process. Among the program strengths were: the development and evaluation of a suite of biological endpoints using the same material; the biological tests represented different levels of biological organization; the tests were conducted under both laboratory and field exposure conditions; tissue residues were examined concurrently with measurements of biological effects; the duration of the study was adequate to evaluate the use of community responses as a benchmark against which other biological responses could be compared; and the development of methods for determining field exposures in the water column and benthic compartments. Limitations of this study were: only one dredged material was evaluated, which constrained certain types of comparisons; the scope of the study put limits on the extent to which any given objective was examined; and the resources allocated to determine field

Table 1
Concentrations of the 10 Selected Contaminants and Two Summary Statistics
for BRH and Reference Sediments (Means \pm Standard Deviations)

Chemical Compound	Sediment* (N)**	
	BRH	REF
Phenanthrene	5,000 \pm 1,800 (15)	85 \pm 17 (12)
Sum of 178 alkyl homologs	28,000 \pm 8,300 (15)	170 \pm 26 (12)
Fluoranthene	6,300 \pm 1,300 (15)	240 \pm 33 (12)
Benzo(a)pyrene	3,900 \pm 970 (15)	250 \pm 28 (12)
Ethylan	4,000 \pm 820 (15)	0 \pm - (12)
PCB as A1254	6,400 \pm 840 (15)	39 \pm 4 (12)
SUM of PAHs	142,000 \pm 30,000 (15)	4,500 \pm 510 (12)
CENTROID of PAHs	232.8 \pm 1.7 (15)	249.2 \pm 1.7 (12)
Copper	2,900 \pm 310 (18)	60 \pm 3 (15)
Cadmium	24 \pm 0.6 (18)	0.23 \pm 0.04 (15)
Chromium	1,480 \pm 83 (18)	50 \pm 15 (15)
Iron	31,000 \pm 2,800 (18)	21,000 \pm 1,400 (15)

* Units are: nanograms/gram dry weight for the organic compounds and the statistic SUM, micrograms/gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

** Number of sediment samples analyzed.

exposures were limited. The last constraint was particularly important because the laboratory-field comparisons required prediction of environmental exposures.

Laboratory-to-Field Comparisons

13. The field verification of laboratory test methods was designed to compare the exposure-response relationships measured in both the laboratory and the field. Exposure for the purposes of this discussion refers to the total dredged material with all of its contaminants. Specific contaminants are used as "tracers" to verify the exposure environment, which is described in terms of BRH dredged material, and to illustrate exposure-response relationships between the laboratory and field. The specific contaminants are

a subset of a comprehensive suite of chemicals analyzed in this study and were selected based upon their environmental chemistry and statistical representativeness. The use of specific contaminants in no way implies a cause-and-effect relationship between contaminant and response.

Residue-Effects Comparisons

14. The relationship between contaminant tissue residues and the biological responses was determined in the laboratory and field for *M. edulis* and *N. incisa*. The approach was to determine specific contaminant residues in the tissues of the organisms as the result of exposure to the whole dredged material at the same time biological responses were being measured. The exposure-residue relationships measured in laboratory experiments indicated that some contaminants in the BRH sediment were biologically available. The relationships between contaminant residues and biological responses do not imply cause and effect, but rather seek to determine the statistical correlation between the residues and responses.

Laboratory and Field Experimental Designs

15. The field verification of laboratory test methods was accomplished by comparing exposure-response relationships measured in the laboratory with those measured in the field. The exposure estimates used in the FVP assume that the dredged material on the mound can be resuspended by various physical processes, such as tidal currents and wind-generated waves. Repartitioning of the contaminants between the dissolved and particulate phases could result as the dredged material is mixed with background material, transported both vertically and horizontally, and then redeposited. These processes create an exposure field in the vicinity of the disposal site that can vary continuously in space and time.

16. The field stations used to study the biological responses were selected along the primary axis of current flow to represent a gradient of potential exposure for the biota. Laboratory exposures were designed to simulate the field conditions. However, because exposure in open marine systems is characterized by highly dynamic temporal and spatial conditions, these conditions cannot be completely replicated in laboratory systems. Consequently,

the approach chosen for this program was to develop laboratory exposure-response data using only general field exposure information and to make qualitative comparisons between the exposure-response relationships.

Laboratory Exposure

17. Laboratory studies constituted a major component of the FVP and can be grouped into three categories: (a) acute toxicity studies on bedded and suspended sediment exposures to 11 marine species for the initial toxicological characterization of dredged material (Rogerson, Schimmel, and Hoffman 1985); (b) determining the applicability and reproducibility of sublethal, chronic test methods for scope for growth (SFG), bioenergetics, adenylate energy charge (AEC), histopathology (HP), sister chromatid exchange (SCE), population, and bioaccumulation responses for use with dredged material; and (c) determining the exposure-response relationships for the sublethal and chronic responses for field verification of laboratory response (Table 2).

18. Two sediments were used to conduct laboratory tests, a reference (REF) sediment and BRH sediment. The REF sediment was collected from the South Reference site in CLIS. The BRH sediment was maintained anaerobically prior to being introduced into the exposure system for the sediment characterization and laboratory documentation phases of the study. In the laboratory studies comprising the field verification portion of the program, BRH and REF sediments were oxidized for 3 days prior to introduction into the exposure system (Nelson et al. 1987).

19. To facilitate laboratory exposures and comparisons between the chronic responses, test systems and exposure designs were developed that permitted subsets of organisms to be sampled and analyzed for SFG, AEC, SCE, HP, and tissue residues from the same experimental unit. Organisms were exposed to constant total suspended sediment concentrations that contained varying amounts of BRH sediment. To achieve the desired suspended sediment exposure gradients, 3-percent slurries of BRH and REF sediments were proportionally mixed, diluted with seawater, and continuously flowed through the exposure chamber (Nelson et al. 1987). For infaunal and epibenthic species, bedded REF sediment was used with suspended sediments in the exposure design. For only bedded sediment exposures, proportional volume/volume mixtures of BRH and REF sediments were used. Chronic responses were measured on a subset of exposed

Table 2

Marine Species and Biological Responses Studied in the FVP*

Species	Program Phase**	Indigenous	Surrogate	Mortality	Bioaccum- ulation	SFG	AEC	SCE	HP	Growth	Repro- duction	Population Growth
Annelids												
<i>Neanthes arenaceodentata</i>	SC	-	+	+	-	-	-	-	-	-	-	-
	LD	-	+	-	-	+	-	-	+	-	-	-
<i>Nephtys incisa</i>	SC	+	-	+	-	-	-	-	-	-	-	-
	LD	+	-	-	+	+	+	+	+	+	-	-
	FV	+	-	-	+	+	+	+	+	+	-	-
Molluscs												
<i>Mytilus edulis</i>	LD	-	+	-	+	+	+	-	+	+	-	-
	FV	-	-	-	+	+	+	-	+	+	-	-
<i>Yoldia limatula</i>	SC	+	-	+	-	-	-	-	-	-	-	-
	LD	+	-	-	-	-	-	-	+	-	-	-
<i>Mulinexa lateralis</i>	SC	+	-	+	-	-	-	-	-	-	-	-
Anthropods												
<i>Ampelisca abdita</i>	SC	+	-	+	-	-	-	-	-	-	-	-
	LD	+	-	+	-	-	-	-	+	+	+	+
	FV	+	-	+	-	-	-	-	-	+	+	+
<i>Mysidopsis bahia</i>	SC	-	+	+	-	-	-	-	-	-	-	-
	LD	-	+	+	-	-	-	-	-	+	+	+
	FV	-	+	+	-	-	-	-	-	+	+	+
Fishes												
<i>Menidia menidia</i>	SC	-	+	+	-	-	-	-	-	-	-	-
<i>Cyprinodon variegatus</i>	SC	-	+	+	-	-	-	-	-	-	-	-
<i>Ammodytes americanus</i>	SC	+	-	+	-	-	-	-	-	-	-	-
<i>Paralichthys dentatus</i>	SC	+	-	+	-	-	-	-	-	-	-	-
<i>Pseudopleuronectes americanus</i>	SC	+	-	+	-	-	-	-	-	-	-	-

* Species or parameter studied (+); not studied (-).

** SC = sediment characterization, LD = laboratory documentation, FV = field verification.

organisms at time intervals specified by the test species and response being measured.

Nephtys incisa

20. *Nephtys incisa* were exposed to BRH and REF sediments in the bedded phase, the suspended particulate phase, and combinations of both phases. Bedded phase exposures ranged from 0-percent BRH (100-percent REF) to 100-percent BRH sediment for up to 56 days. A suspended sediment proportional diluter was used to mix small quantities of concentrated sediment slurries from a sediment dosing system with filtered seawater to produce dilute sediment suspensions. The diluter combined slurries of both sediments proportionally to maintain the same concentration of suspended sediment while producing different ratios of the two sediments. Suspended sediment exposures ranged from 0 mg/l to 200 mg/l of BRH sediment.

Mytilus edulis

21. *Mytilus edulis* were exposed continuously to suspensions of REF and BRH sediments singularly and as proportional mixtures for periods up to 28 days (Nelson et al. 1987). The experimental design included exposures of 100-, 50-, 30-, 10-, and 0-percent BRH sediment. While the percentage mixtures varied between treatments, a total suspended sediment concentration of 10 mg/l was maintained in all laboratory exposures, resulting in nominal exposures of 10, 5, 3, 1, and 0 mg/l of BRH suspended sediment. Because *M. edulis* is a filter feeder, each exposure chamber was equipped with a transmissometer-microprocessor feedback loop to maintain constant exposure conditions. This system controlled the dosing valves that proportionally mixed concentrated BRH and REF sediment slurries from a sediment dosing system with filtered seawater to produce the desired suspended sediment concentrations (Sinnott and Davis 1983).

Field Exposure

Nephtys incisa

22. Site-specific physical and chemical data were used to estimate BRH exposure at the FVP stations. During disposal and for 1 to 2 months post-disposal, water chemistry data were used to estimate the BRH suspended sediment concentration 1 m above the bottom for the FVP stations (Nelson et al. 1987). Suspended sediment and hydrographic data indicated a 10x enrichment

from 1 m above the bottom to the sediment/water interface. Based upon these data, exposures at the sediment/water interface were estimated to range from 7 to 13 mg BRH/l at the FVP stations. Sediment/water interface exposures estimated from resuspension of surficial sediments indicated much higher concentrations. A maximum upper bound, which assumes that all of the suspended solids are BRH material from the disposal mound, was calculated to be 100 mg BRH/l suspended sediment under quiescent conditions and up to 300 mg BRH/l under storm conditions. A more probable estimate assumes that sediments resuspended at each station are the source of contaminants for the suspended solids. This estimate predicts maximum exposure values at the FVP stations of 41 mg/l at station 200E, 12 mg/l at 400E, and 4 mg/l at 1000E for quiescent conditions. These values increase to 123 mg/l at station 200E, 37 mg/l at 400E, and 6 mg/l at 1000E for storm conditions (Table 3).

23. A final method for estimating field exposures assumed that tissue concentrations in *N. incisa* are directly related to exposure concentrations. This relationship was derived from laboratory exposure-residue relationships. If this relationship applies in the field, it may be used to test the accuracy of the exposure predictions. The estimates of field exposures to BRH sediment (milligrams per litre) suspended at the sediment/water interface, based on PCB concentrations in field-collected *N. incisa*, ranged from 2 to 12 mg/l at REFS, from 43 to 88 mg/l at station 1000E, and from 95 to 131 mg/l at station 400E during the period June to September 1983.

24. Assuming that field exposures were probably due to initial dispersion of BRH sediments during disposal and subsequent resuspension and movement of sediments from the dredged material mound, a combination of estimates seems appropriate. The estimate based on water chemistry predicts exposures of at least 7 mg/l at the sediment/water interface at all FVP stations during disposal activities in CLIS. The worst-case resuspension estimate predicts exposures of up to 100 mg/l in the vicinity of the disposal mound. These estimates (7 to 100 mg/l) agree well with those predicted by the tissue concentration-exposure concentration relationship (12 to 131 mg/l). The laboratory exposures for infaunal and epifaunal organisms ranged from 0 to 200 mg BRH/l suspended sediment, which includes the estimated range of exposures in the field.

Table 3
Range of Field Exposures (mg/l BRH Sediment) at the Disposal Site*

Method of Estimation	Sediment/Water Interface, Station No.				Water Column, Station 400E	
	200E	400E	1000E	REFS	Jun 83	Aug 83
Water chemistry						
PCB	--	7-11	-	--	0.7-1.1	0.1-0.2
Copper	--	7-13	-	--	0.7-1.3	0.3-0.6
Tissue residues (PCB)	NA**	95-131	43-88	2-12	0.8-1.4	0.1-0.7
Suspended sediment						
Maximum estimate						
Background	10-100	10-100	NA	NA	NA	NA
Storm	30-300	30-300	NA	NA	NA	NA
Suspended sediment and sediment chemistry						
Probable estimate						
Background	37-41	5-12	1.6-2.0	--	NA	NA
Storm	110-123	9-37	4.8-6.0	--	NA	NA

Note: Estimated from suspended sediment, water and sediment chemistry, and tissue residue data for sediment/water interface and the near-bottom water column.

* Estimates from June-September 1983.

** NA = not applicable.

Mytilus edulis

25. *Mytilus edulis* were deployed from subsurface buoys at stations CNTR, 400E, 1000E, and REFS, 1 m above the bottom, for 28-day periods throughout the predisposal and postdisposal portions of the study. Field BRH exposures were estimated from water column measures of PCB and copper and by substituting the field PCB tissue residue concentrations into a laboratory-derived equation describing the residue-exposure relationship for mussels (Nelson et al. 1987). The concentration of BRH sediment that would be necessary to achieve the observed field tissue residue values was then calculated from the laboratory equation.

26. The predicted water column exposures for each station at 1 m above the bottom and for each collection date had a distinct spatial and temporal

pattern. Spatially, the data indicate elevated water column exposures on and around the mound compared to the 1000E and REFS stations.

27. Temporally, the estimated water column BRH exposures decreased with increasing time from disposal. A maximum exposure range of 1.3 to 0.7 mg/l of BRH suspended sediment was estimated for the 400E station within the first 2 weeks after disposal. These concentrations decreased to 0.6 to 0.3 mg/l within 8 weeks of disposal and continued to decrease to levels similar to those at the REFS station within 12 to 15 weeks after the cessation of disposal operations. Thus, water column exposures at 1 m above the bottom resulting from disposal activity persisted for only 2 months, after which exposures were comparable at all stations.

PART II: BIOLOGICAL RESPONSES IN THE LABORATORY AND FIELD

Scope for Growth

Laboratory documentation

28. The purpose of the laboratory documentation portion of the FVP was to assess the sensitivity, reproducibility, and variability associated with the scope for growth index for *M. edulis*. The SFG was determined by measuring four physiological parameters (clearance rate, absorption efficiency, respiration rate, and ammonia excretion rate) on individual mussels after exposure to BRH suspended sediment. Two independent experiments of 28 days duration were completed, each with BRH exposures of: 100-percent BRH sediment, 100-percent REF sediment, and a 50/50 mix of BRH and REF sediment.

29. The results of the first experiment indicated an inverse relationship between SFG and BRH exposure concentration. The SFG values for the 100-percent REF, 50-percent REF/BRH, and 100-percent BRH treatments were 2.53, -2.32, and -3.63 J/hr, respectively. These data indicated that the SFG index was sufficiently sensitive to detect physiological changes in the mussel after exposure to BRH sediment. The second experiment indicated the same pattern of SFG response to BRH sediment with mean SFG values of 10.22, 0.51, and -1.07 J/hr for the 100-percent REF, 50/50-percent REF/BRH, and 100-percent BRH treatments, respectively. The absolute SFG values were not the same between experiments because of seasonal variability in temperature and food supply. The important point is that the SFG values showed the same relative response in both BRH experiments. Therefore, the general conclusion of these two experiments was that the SFG index, when used with *M. edulis*, was both a sensitive and reproducible measure for assessing the physiological effects of exposure to BRH sediment.

30. The suspended sediment exposure system designed for the laboratory documentation studies lacked a feedback control for maintaining a constant suspended sediment concentration, which resulted in a twofold excursion in exposure concentration (Nelson, Black, and Phelps 1985). This is important when studying a filter-feeding bivalve mollusc, such as *M. edulis*, which can filter large volumes of water efficiently. As a result, the exposure design used in the laboratory documentation phase of this study is not recommended

and was replaced in the field verification phase of the program with a microprocessor-controlled exposure system.

Field verification

31. This portion of the FVP was designed to: (a) establish the relationship between BRH exposure and the SFG response of mussels separately in the laboratory and the field, and (b) determine the degree of comparability between these exposure-response relationships. A laboratory dosing system was developed to expose mussels to a relatively constant concentration of 10 mg/l of suspended sediment, while the proportion of BRH and REF sediment comprising that total varied between treatments. The actual treatment concentrations of BRH suspended sediment used in this study were 0, 1.5, 3.3, 5.0, and 10 mg/l. Two experiments were completed. An initial experiment, consisting of BRH exposures of 0, 5.0 and 10 mg/l, was stopped after 14 days because severe reductions in SFG were observed in the 5- and 10-mg/l BRH treatments. A second experiment, with treatment concentrations of 0, 1.5, and 3.3 mg/l BRH sediment, was conducted for 28 days, which was comparable to the length of exposure for mussels deployed under field conditions.

32. The exposure-response relationship for *M. edulis*, measured in the laboratory and field, is illustrated in Figure 3. The laboratory results indicate a negative response to BRH exposure. The lowest BRH concentration tested in the laboratory, 1.5 mg/l, produced a 45-percent decrease in scope for growth compared to the REF control, which increased to greater than 80 percent at exposure concentrations ≥ 3.3 mg/l. The field exposures were considerably less than those in the laboratory, reaching a maximum of 1.4 mg/l during and within the first month after disposal (Table 3), after which time the field exposures in the water column approached background conditions. The scope for growth index showed a clear response to BRH exposure in the field, which was consistent with that predicted from the laboratory data (Figure 3). These data lead to the conclusion that the exposure-response relationships between the laboratory and field were comparable and that there was a field verification of the laboratory scope for growth response.

Residue-effects comparisons

33. The residue-effect relationships determined in this study were never intended to determine cause and effect of individual compounds. However, relationships between individual contaminants and biological effects present strong evidence that observed reductions in SFG were related to

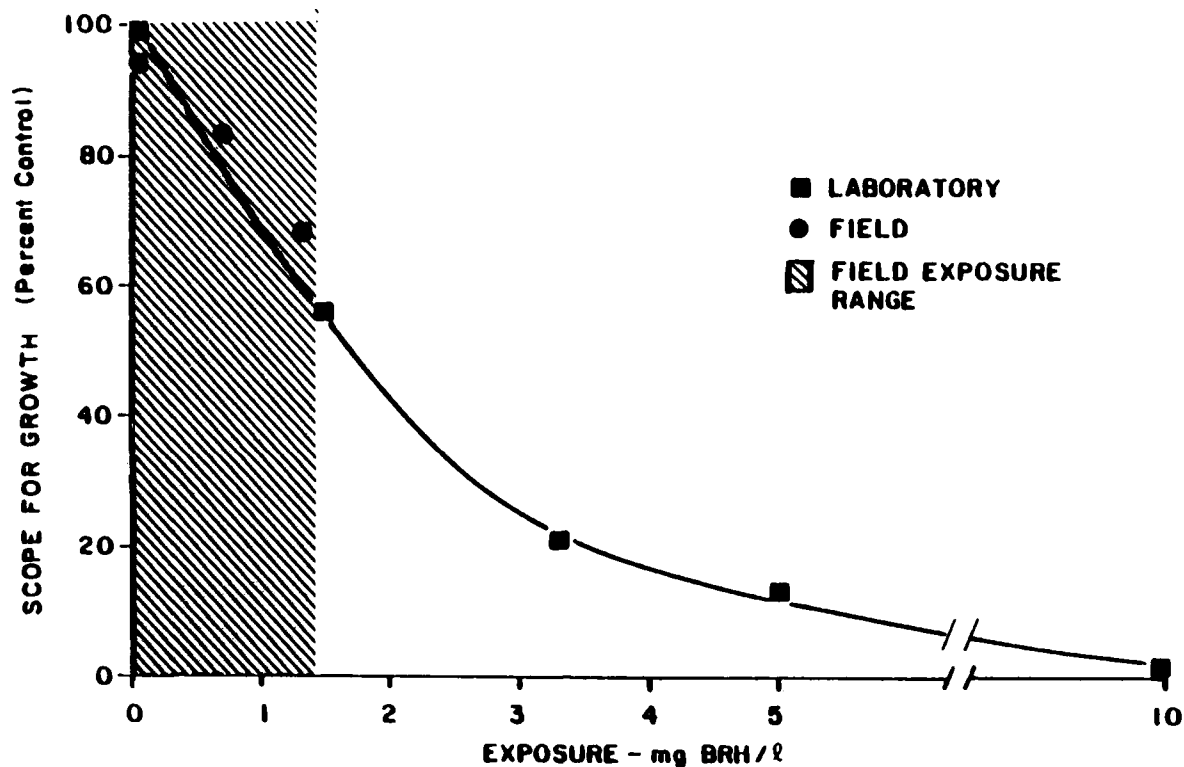


Figure 3. Exposure-response relationship for SFG in *M. edulis* measured in the laboratory and field

exposure to BRH sediment. Analysis of tissue residues and SFG effects in the laboratory experiments indicated a significant inverse relationship between SFG and most inorganic contaminants, including copper and cadmium, and for heavier molecular weight organic compounds, such as PCB, benzo(a)pyrene, and fluoranthrene. The laboratory data show that: (a) increased BRH exposure concentration resulted in elevated tissue residues of the inorganic and organic compounds present in the BRH material, and (b) the subsequent inverse relationship between tissue residue concentrations and SFG of mussels indicated that BRH exposure was responsible for the adverse physiological effects.

34. In the field, the relationship between residue and effects was not as apparent as it was in the laboratory, because BRH field exposures were lower than those in the laboratory. Statistical analysis of residue data from both the laboratory and the field indicated that the residue data from only one field collection, 2 weeks postdisposal, were similar to the laboratory

residue concentrations. All other field residue data were most similar to laboratory treatments with no BRH material.

Bioenergetics

Laboratory documentation

35. Physiological responses measured in *N. incisa* juveniles included growth, respiration, ammonia excretion rate, oxygen-nitrogen ratios (O:N), and burrowing activity. From these responses, cumulative energy for production and maintenance, net growth efficiency, and scope for growth were calculated. The 10-day exposures used in these studies included bedded and suspended sediments individually or in combination with varying percentages of BRH and REF sediments.

36. All the biological responses with the exception of the calculated O:N ratios showed an exposure-response relationship to BRH sediment. The precision of each response measured within an experiment exhibited a coefficient of variation about the mean value of less than 28 percent. Reproducibility between experiments was excellent. Measured physiological values from the same treatment between two replicate experiments were not significantly different from each other (Johns, Gutjahr-Gobell, and Schauer 1985).

37. The results from these studies are as follows. There were no acute toxicity responses in either *N. incisa* or *Neanthes arenaceodentata* in any of the 10-day exposures to BRH sediments. In the bedded sediment experiments, all biological responses, except the O:N ratio, were significantly affected in 100-percent BRH sediment with *N. incisa*, while only scope for growth was affected in *Neanthes*. Growth and net growth efficiency in *N. incisa* were reduced 60 and 40 percent, respectively, in the two experiments at the 50-percent BRH sediment concentration. Scope for growth in *Neanthes* decreased 11 percent in the 50-percent BRH sediment.

38. In tests with simultaneous suspended and bedded sediment exposures, the presence of BRH sediment generally resulted in a decrease in physiological response, though the magnitude varied. The greatest magnitude and most consistent impact occurred when both the bedded and suspended sediments consisted entirely of BRH sediments. All responses in *N. incisa*, except the O:N ratios, were significantly affected in the latter treatment. Only net growth

efficiency and scope for growth were affected in *Neanthes*. Other exposure combinations produced an intermediate range of responses.

39. The laboratory documentation studies demonstrate that physiological responses can be measured in the laboratory for infaunal species; the magnitude of the change in these responses was directly related to the concentration of BRH sediment; and the precision and reproducibility of the response were excellent.

Field verification

40. The laboratory component of field verification was conducted with chronic (28- to 42-day) exposure of *N. incisa* to suspended sediments. The primary responses measured in the laboratory were growth, respiration, excretion, production, and net growth efficiency. The results show that all responses were affected significantly at 100-mg/l BRH suspended sediments, which was the lowest BRH sediment concentration tested. However, a 50-percent decrease in response (EC50) was estimated for growth, production, and net growth efficiency at 25, 30, and 50 mg/l BRH, respectively (Johns and Gutjahr-Gobell 1988).

41. Field exposures were estimated from empirical physical and chemical data and from tissue residues of *N. incisa* collected at the disposal site. Field responses consisted only of respiration and excretion rates measured on *N. incisa* collected at stations 200E, 400E, 1000E, and REFS. The temporal scale of the field program included a single predisposal and eight postdisposal sampling periods over 3 years. *Nephtys incisa* exposed and collected at 200E (100 mg BRH/l) and 400E (50 mg BRH/l) had significantly lower respiration and excretion rates than worms exposed and collected at REFS and 1000E during seasons when the seawater temperatures were between 12° and 21° C (Figures 4 and 5). This spatial pattern persisted throughout the summers of 1983, 1984, and 1985. At temperatures below 12° C, *N. incisa* metabolic activity enters a state of quiescence and the spatial response pattern disappeared (Johns and Gutjahr-Gobell 1988).

42. The exposure-response relationships for respiration and excretion in *N. incisa* measured in the laboratory and field are presented in Figures 4 and 5. The laboratory respiration rates were markedly increased over controls by exposure to ≥ 100 mg BRH/l. In contrast, field respiration rates were significantly decreased at ≥ 100 mg BRH/l (stations 200E and 400E). Field *N. incisa* were exposed to both bedded and suspended sediments, while laboratory

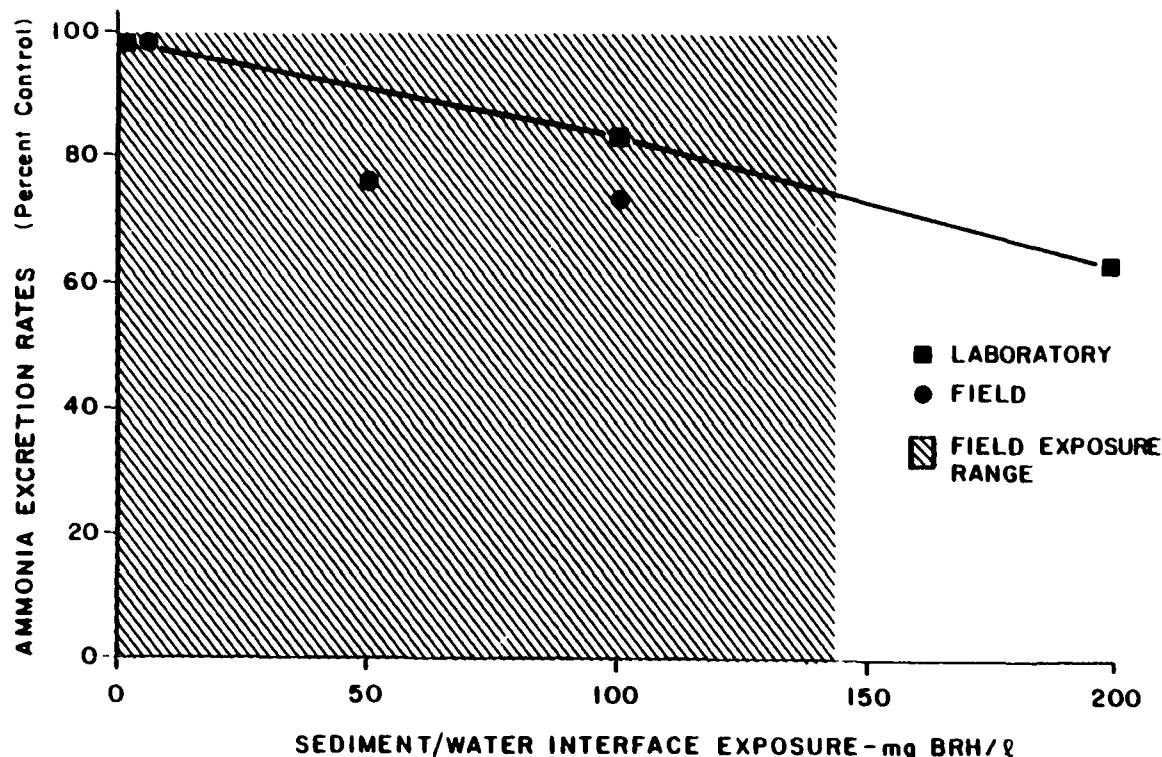


Figure 4. Exposure-response relationship for ammonia excretion rates in *N. incisa* measured in the laboratory and field

studies provided exposure only to suspended sediment. Excretion rates in the laboratory and field decreased significantly with increasing BRH exposure, and laboratory and field excretion rates concurred over the range of field exposures.

Residue-effects comparisons

43. In the laboratory, there was a strong inverse correlation between 9 of 11 selected contaminants and growth, production, and net growth efficiency; that is, when tissue residues increased, the magnitude of the bioenergetic responses decreased. Weight-specific respiration in the laboratory was inversely correlated with cadmium, while weight-specific excretion was inversely correlated with the lower molecular weight PAHs. Similar analyses conducted in the field indicate that respiration and excretion values did not correlate with field tissue residues. The exceptions include the PCBs, which had a linear correlation of -0.61 with respiration and -0.71 with excretion, and benzo(a)pyrene and iron, which were significantly correlated (-0.61 and -0.69, respectively) with excretion.

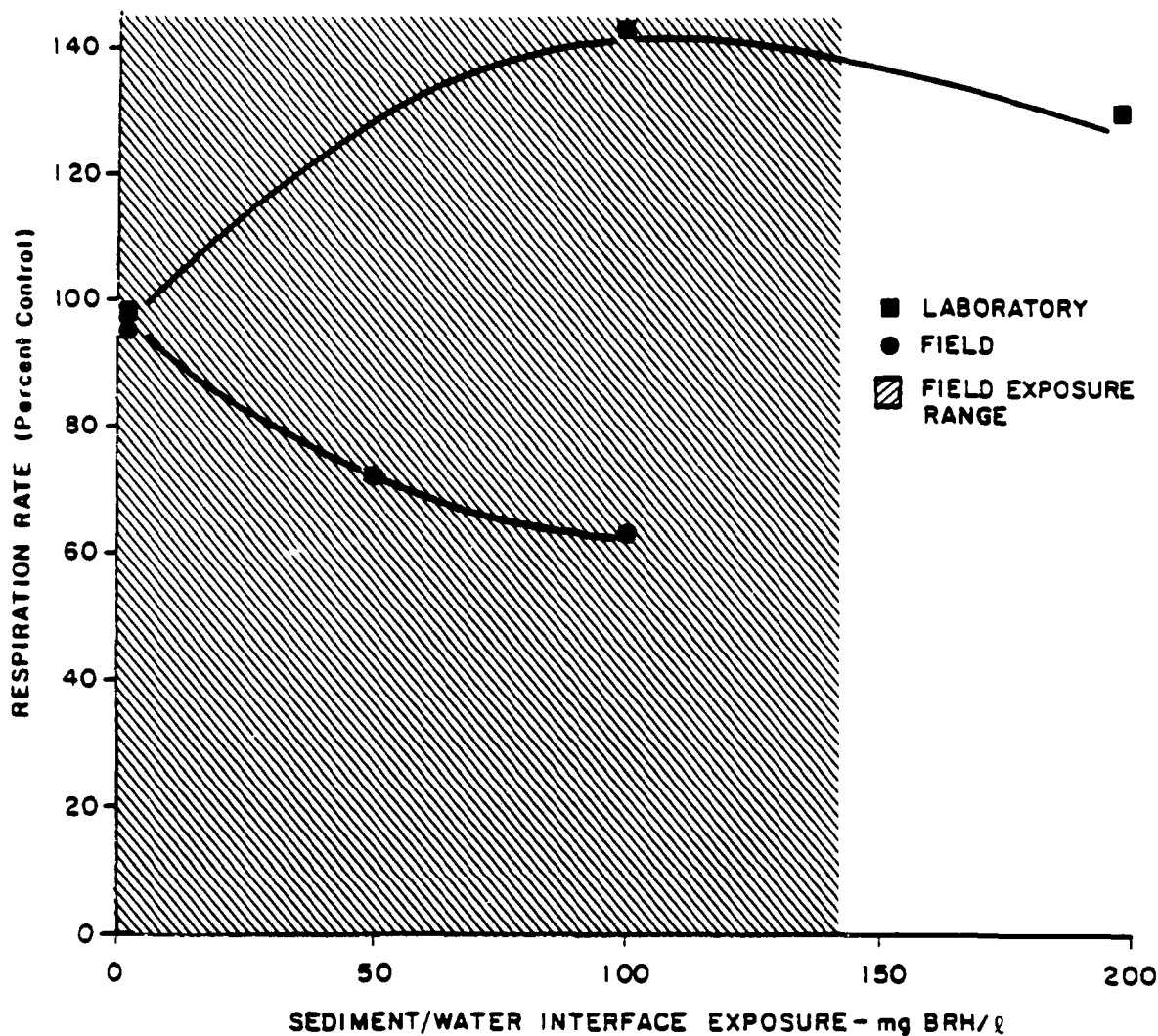


Figure 5. Exposure-response relationship for respiration rate in *N. incisa* measured in the laboratory and field

Adenylate Energy Charge

44. Atkinson and Walton (1967) proposed AEC as a measure of energy potentially available from the adenylate system for cell metabolism. The AEC, defined as $(ATP + 1/2 ADP)/(ATP + ADP + AMP)$, has a maximum value of 1.0 when all adenylate is in the form of ATP, and a minimum value of 0 when all adenylate is in the form of AMP (Atkinson and Walton 1967).* The energy

* ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate.

charge is considered important in the control of key catabolic and anabolic pathways (Atkinson 1971). Values of energy charge correlate with physiological condition: energy charges between 0.8 and 0.9 are typical of organisms that are actively growing and reproducing usually under optimal environmental conditions; values in the range of 0.5 to 0.7 have been observed in organisms that are stressed and whose growth and reproductive rates are reduced (Atkinson 1971; Chapman, Fall, and Atkinson 1971; Ivanovici 1980). Values below 0.5 have been associated with irreversible loss of viability under detrimental conditions (Skjoldal and Bakke 1978).

45. In this report the responses, of the adenine nucleotide pool (the sum of all the adenine pools) and adenylate energy charge to stress are examined. The central role of adenine nucleotides in energy transformation and in metabolic regulation suggests their potential usefulness as indicators of sublethal stress. These responses were measured in the mussel *M. edulis* and the polychaete *N. incisa* exposed to BRH dredged material, both in the laboratory and the field.

Mytilus edulis

46. Laboratory documentation. Laboratory documentation focused on the technical development and application of measuring adenine nucleotides in the adductor muscle of *M. edulis* and the whole body of *N. incisa*. Modification of the methodology was required to conduct adenine nucleotide measurements in the laboratory and field. This included examination of anesthetization and handling techniques, modification of extraction techniques, and determination of recovery efficiencies. Because of limitations in the exposure designs, a definitive assessment of the adenine nucleotide responses to BRH sediment was not determined (Zaroogian et al. 1985).

47. Field verification. Adenine nucleotides were measured in the adductor muscle tissue of *M. edulis* in two laboratory experiments and predisposal and postdisposal in the field. *Mytilus edulis* exposed in the laboratory exhibited stress as evidenced by reduced scope for growth, clearance rates, and shell growth (Nelson et al. 1987). It might be expected that under stress conditions and reduced food intake, the adenine nucleotide pools would be affected. However, of the nucleotide variables examined, ATP, ADP, AMP, AEC, and the total adenine pool, only the latter showed a predictable response to BRH exposure. The AEC measured in *M. edulis* did not respond in a predictable manner to BRH exposure in the laboratory (Zaroogian et al. 1988).

48. The exposure-response relationship for AEC in *M. edulis* for the laboratory and field is illustrated in Figure 6. Because AEC did not show a quantitative and predictable response to exposure to BRH sediments in the laboratory, it was neither a useful indicator nor a predictor of stress. Therefore, laboratory results could not be used to predict stress in exposed field organisms.

49. The exposure-response relationship for the total adenine pool in *M. edulis* in the laboratory did show a consistent response to exposure to BRH sediment when the replicates of two experiments were pooled (Figure 7). It is also evident that the field exposure to *M. edulis* was well below the laboratory concentrations producing the maximum response in the total adenine pool. From these data an estimated BRH exposure ≥ 5 mg/l would be required to cause a ≥ 20 -percent decrease in the total adenine pool. The estimated maximum exposure concentration in the field, 1.4 mg/l, is similar to the lowest laboratory

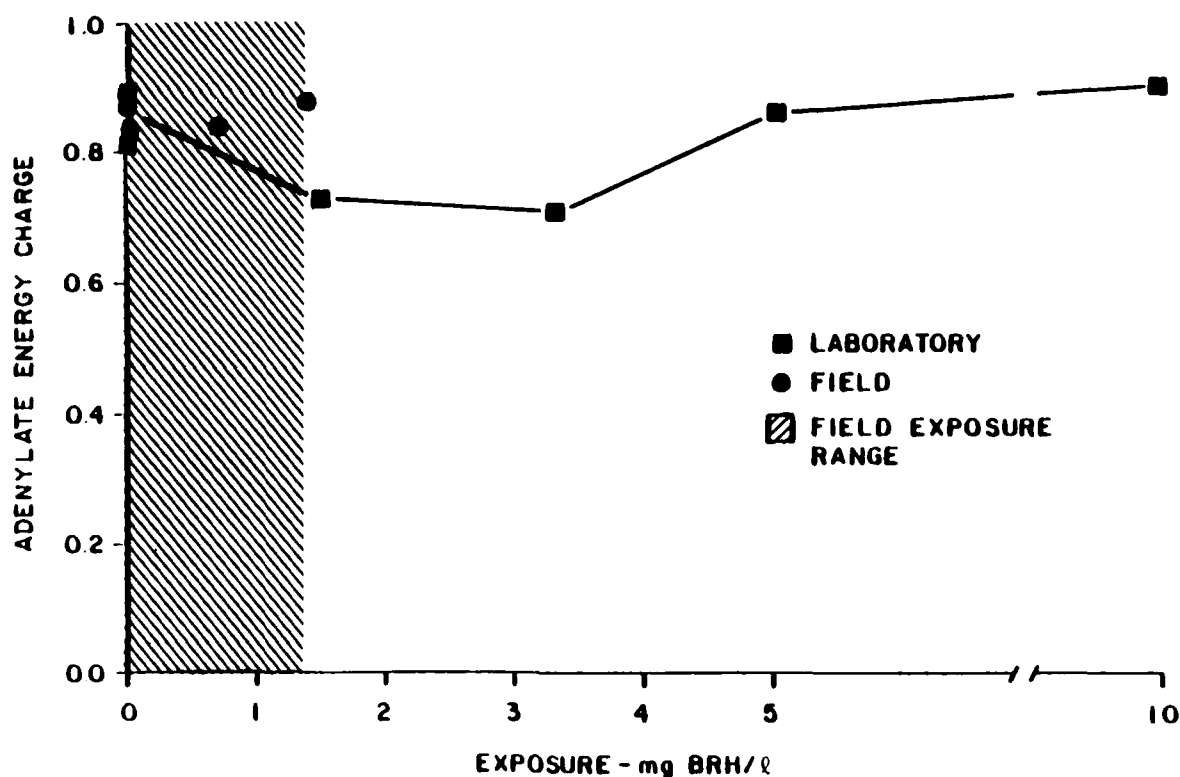


Figure 6. Exposure-response relationship for AEC in *M. edulis* in the laboratory and field

exposure (1.5 mg/l), which did not significantly affect the adenine pool in mussels. Based upon the relationships between exposure and response illustrated in Figure 7, one would not expect to detect significant changes in the total adenine pool in *M. edulis* exposed in the field. Thus, the field-exposed organisms were below the threshold for effects as determined in the laboratory, and showed a response pattern that was consistent with laboratory-exposed organisms.

50. Residue-effects comparisons. Correlation analysis was used to determine whether any relationships existed between AEC, concentrations of ATP, ADP, AMP, total adenylate nucleotide pools, and tissue concentrations of the selected 10 chemicals and two summary statistics for PAHs. In the laboratory, significant correlations existed between total adenine pool and sum of 178 alkyl homologs, fluoranthene, benzo(a)pyrene, sum of PAHs, PCB as Aroclor 1254 (A1254), ethylan, copper, and cadmium. This represents a broad spectrum of chemicals, including several classes of organic compounds and

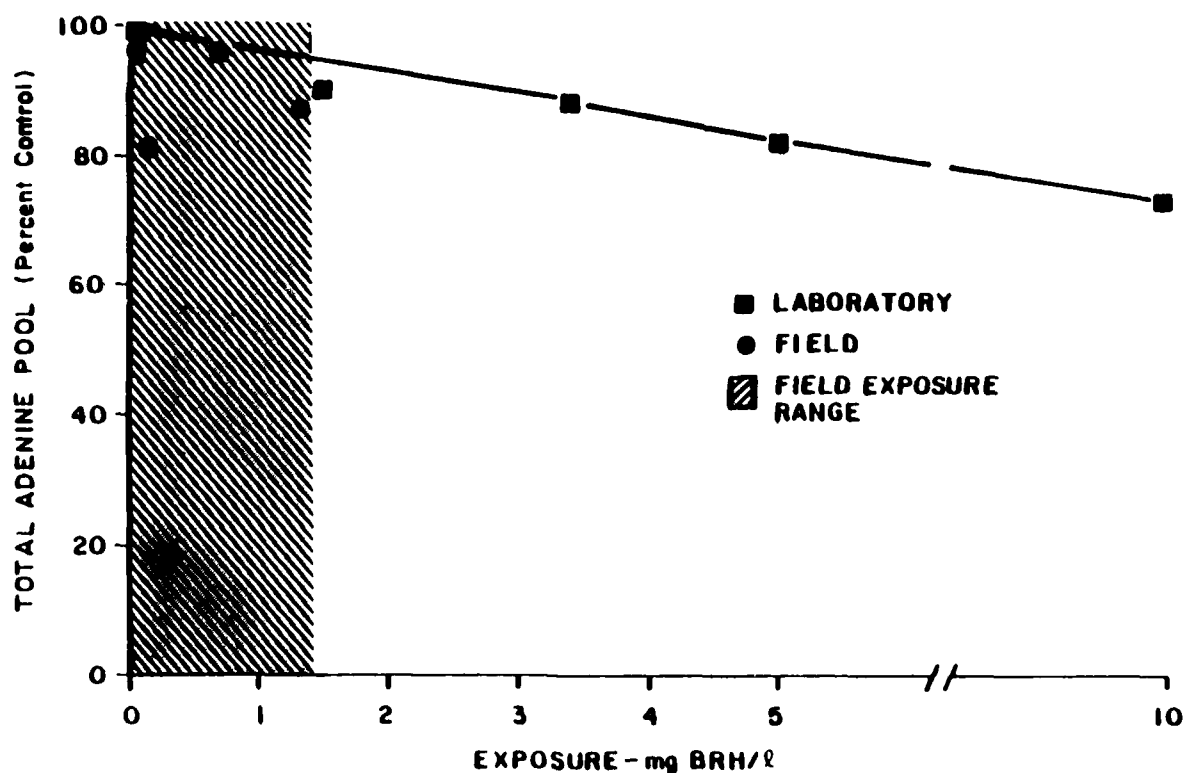


Figure 7. Exposure-response relationship for total adenine pool in *M. edulis* in the laboratory and field

metals. It is interesting to note that in other contaminant biological evaluations conducted during these same experiments, significant correlations also existed between scope for growth responses and the same broad spectrum of chemicals (Nelson et al. 1987). Thus, these two measures of energy utilization suggest that these chemicals may directly or indirectly affect energy flow and utilization in *M. edulis*.

51. In contrast to the laboratory results, no residue-effects relationships were measured in the field. The contaminant residues in field mussels were most similar to those of mussels exposed to reference sediment in the laboratory.

Nephtys incisa

52. Field verification. Adenine nucleotides were measured in whole worms from all treatments at all sampling times in three laboratory experiments. Exposure conditions ranged from 0 to 200 mg BRH/l for 42 days. Of the five variables considered (ATP, ADP, AMP, AEC, and the total adenine nucleotide pool), none exhibited a response to BRH exposure. This indicates that AEC and the four associated variables had a response threshold in excess of 200 mg/l BRH suspended sediments (Zarogian et al. 1988). This is surprising since reduction in growth, increase in respiration rate, and exposure-related reduction in net growth efficiency were reported for worms from the same exposure treatments (Johns and Gutjahr-Gobell 1988).

53. The exposure-response relationships for AEC and total adenine nucleotide pools in *N. incisa* for the laboratory and field are illustrated in Figures 8 and 9. It is evident that neither AEC nor the total adenine pool showed an exposure relationship over the range of BRH exposures examined in the laboratory.

54. Because of difficulties with adenylate extraction from *N. incisa* during the first several months of field sampling, only the data beginning with September 1983 could be compared with the laboratory data to establish the degree of field verification. The results of field AEC and total adenine nucleotide analyses are included with the laboratory data in Figures 8 and 9.

55. During September 1983, differences were determined for AEC and the total adenine nucleotide pool concentrations in *N. incisa* found on and off the disposal mound. Although differences were detected, the AEC values were indicative of nonstressed organisms and thus do not constitute biological

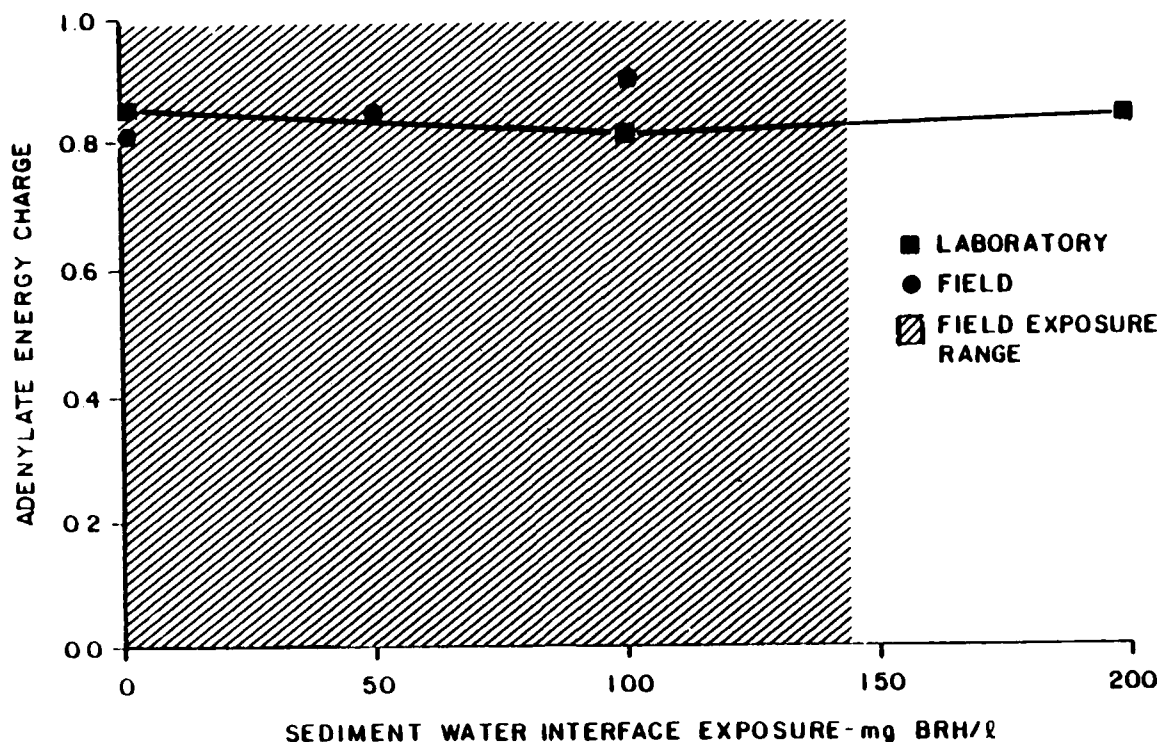


Figure 8. Change in AEC in *N. incisa* exposed to BRH suspended sediment in the laboratory and field

significance. This is consistent with laboratory results where no differences were reported among treatments for any of the adenine nucleotide measurements.

56. The AEC response reported in the field for September 1983 coincides with maximum estimated field exposures as evidenced by the highest PCB tissue residue concentrations measured in *N. incisa* in the field. This difference between laboratory and field responses may be related to the difference in the duration of exposure (6 weeks in the laboratory versus 16 weeks in the field). The short duration of laboratory experiments with *N. incisa* in the FVP was cited as a potential limitation in the cytogenetic studies (Pesch et al. 1987). However, while there were differences in the field responses, the fact remains that these differences were not biologically significant.

57. Residue-effects comparisons. In the laboratory, significant correlations existed between the following: adenine nucleotide pool and phenanthrene; sum of 178 alkyl homologs, benzo(a)pyrene, and sum of PAHs; ATP and phenanthrene, benzo(a)pyrene, and sum of PAHs; ADP and phenanthrene, benzo(a)pyrene, sum of PAHs, and PCB as A1254; and AEC and phenanthrene and

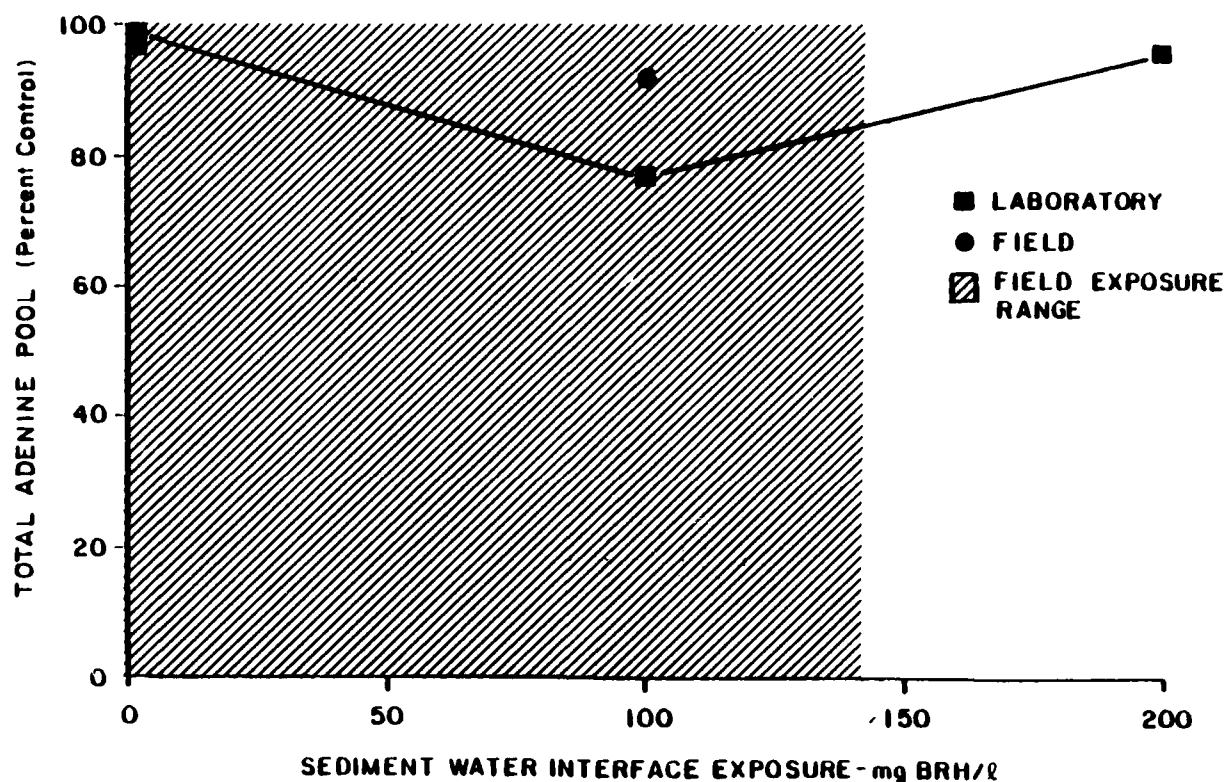


Figure 9. Percent change in adenine nucleotides in *N. incisa* exposed to BRH suspended sediments in the laboratory and field

benzo(a)pyrene. This represents a narrow spectrum of chemicals; 12 of 13 significant correlations were with PAHs. In contrast, the physiological measure of net growth efficiency was inversely proportional to a broad spectrum of chemicals, including several classes of organic compounds plus metals in these same laboratory experiments (Johns and Gutjahr-Gobell 1988). The importance of these correlations is difficult to assess since there were no exposure-response relationships for any of the adenine nucleotides in the laboratory.

58. A direct correlation between tissue concentrations of PAHs and a biological response cannot always be expected. For high molecular weight PAHs it is not the parent PAH compounds that are biologically damaging. Rather, the damage is associated with metabolic products. PAHs are metabolized by the mixed function oxidase (MFO) system. Polychaetes are known to have active, inducible MFO systems (Lee and Singer 1980, Lee et al. 1981). Induction of these enzymes takes weeks to months (Fries and Lee 1984). It is during maximum activity of the MFO system that maximum biological effect would be expected. There were no correlations between tissue concentrations of PAHs

and any measure of the adenine nucleotide responses in the field. This lack of correlation may be attributable to potentially induced MFO activity in field-collected worms.

Sister Chromatid Exchange

Laboratory documentation

59. The SCE response has been recommended for environmental application by the US Environmental Protection Agency Gene-Tox Program (Latt et al. 1981). Several studies have shown that SCE is a more sensitive method for detecting mutagens and carcinogens than the traditional chromosome and chromatid observations (Perry and Evans 1975, Solomon and Bobrow 1975, Bloom 1978). The application of SCE to polychaetes has created a new tool that is both relevant and practical for studying genetic problems in marine environments (Pesch, Pesch, and Malcolm 1981).

60. The SCE technique was applied to *N. incisa*, an infaunal polychaete dominant in the benthic community at the CLIS disposal site. The laboratory phase was replicated three times with *N. incisa* using a randomized complete block design. When the data from all three replicate tests were pooled and statistically analyzed, the frequency of SCE response in *N. incisa* exposed to suspended BRH sediment flowing over bedded REF sediment was significantly higher than all other treatments (Pesch et al. 1987). Differences in SCE response among the laboratory replicate tests suggest that one or more factors important in SCE induction under laboratory conditions were not controlled (Pesch et al. 1987). Preconditioning of worms and thus the induction of MFO activity before their use in the laboratory BRH experiment was the most likely uncontrolled variable in this study. Clearly, the application of SCE to *N. incisa* needs further development before it can be recommended for routine use.

Field verification

61. Field SCE data are available for comparison with laboratory SCE data for three sampling dates: 6 June 1983 (T + 3 weeks), 26 August 1983 (T + 14 weeks), and 13 December 1983 (T + 28 weeks) (Pesch et al. 1987). Only the August field data showed an increased SCE frequency. The elevated SCE frequency in field organisms collected in August declined when the worms were held in clean laboratory conditions, but increased to field levels when the worms were exposed to BRH sediments. Thus, there is a positive correlation

between an increased frequency of SCE response and the presence of BRH sediments. When an SCE response to contaminated sediment was elicited in *N. incisa*, the response was comparable in both the laboratory and the field.

Residue-effects comparisons

62. This objective was addressed by correlating tissue concentrations of 10 chemicals with SCE response. Only two of these chemicals, benzo(a)pyrene and chromium, are known mutagens. Benzo(a)pyrene is not a direct-acting mutagen and, therefore, a correlation with SCE was not expected and did not occur. Although chromium, a known direct-acting mutagen, showed a significant correlation with SCE, the significance of this is obscure, as only hexavalent chromium is known to be a mutagen. Hexavalent chromium was not found in this study but is present in Narragansett Bay and may be present at the FVP disposal site (Bickford 1975). The relationship between sediment concentrations of chromium and SCE response in exposed organisms deserves further research.

Histopathology

Mytilus edulis

63. Laboratory documentation. Histopathological changes were detected in the female reproductive tract and cardiovascular systems of *M. edulis* exposed to suspended BRH sediments. Reproductive tract changes included basophilic ova that show a loss of nuclei, normal cytoplasm, and vitelline membrane, probably resulting from the combination of spawning condition and BRH contaminant stress. The cardiovascular effects were characterized by pedunculated growths arising from the auricle, ventricle, or pericardial walls. The degree of reproducibility of the histological changes was acceptable considering the subjective nature of the endpoint and sample sizes examined (Yevich et al. 1986).

64. Field verification. *Mytilus edulis* exposed continuously in the laboratory to suspended BRH sediments exhibited histopathological changes in the reproductive tract, gills, kidney, and gastrointestinal tract. Minor changes were observed in the byssus organ. The incidence of histopathological change noted within a given organ system was related to the concentration of BRH sediment. The gills were the most severely affected, with pathology incidences of 80 to 100 percent at exposure concentrations of 1 to 3 mg/l of BRH

sediment. The gastrointestinal tract showed incidences of pathology of 7, 50, and 65 percent at exposures of 3, 5, and 10 mg/l BRH sediment, respectively. Reproductive effects were also related to exposure with incidences of 20 and 50 percent at 3 and 10 mg/l BRH sediment, while the incidence of kidney lesions ranged from 7 to 25 percent at exposures of 3 and 10 mg/l BRH sediment, respectively. In the laboratory documentation studies with *M. edulis*, employing somewhat different BRH sediment exposures, histopathological effects were reported for the female reproductive tract and the heart (Yevich et al. 1986).

65. The exposure-response relationships for histopathologies of the gastrointestinal tract and gills in *M. edulis* measured in the laboratory and field are illustrated in Figures 10 and 11. The laboratory results indicate that the incidence of pathologies in these organs is directly related to exposure to BRH suspended sediment. The response threshold for gill pathology is ≥ 1.5 mg BRH/l; for gastrointestinal pathology, the threshold concentration is ≥ 5.0 mg BRH/l suspended sediment.

66. Examination of the field results revealed that no pathology was observed in any of the seven organ systems examined from *M. edulis* deployed at the FVP disposal site station. At 2 months postdisposal, the exposure of *M. edulis* to BRH sediment was similar to the reference site and predisposal conditions. However, during and immediately after disposal, maximum BRH suspended sediment exposures were estimated to be 1.4 mg/l, well below the response threshold for all pathologies except those reported for the gills.

67. The 80-percent incidence of gill pathology in the laboratory at 1.5 mg/l would have indicated a high probability of pathology in the field at an estimated 1.4 mg/l exposure. This lack of concurrence for a single field sampling period is most likely a result of differences in the total integrated exposure duration between the laboratory and field and the time to elicit a response. Unlike laboratory studies, where exposures were continuous, field exposures are highly variable due to changing current velocities and wind-driven waves that affect resuspension of deposited sediments. Therefore, the total integrated exposure (i.e., duration times concentration) in the field was most likely less than one would expect from a temporally discrete estimate of the magnitude of exposure. This is particularly important for pathology since the exposure duration required to elicit structural changes in tissues is greater than that required for physiological changes (e.g., SFG). This

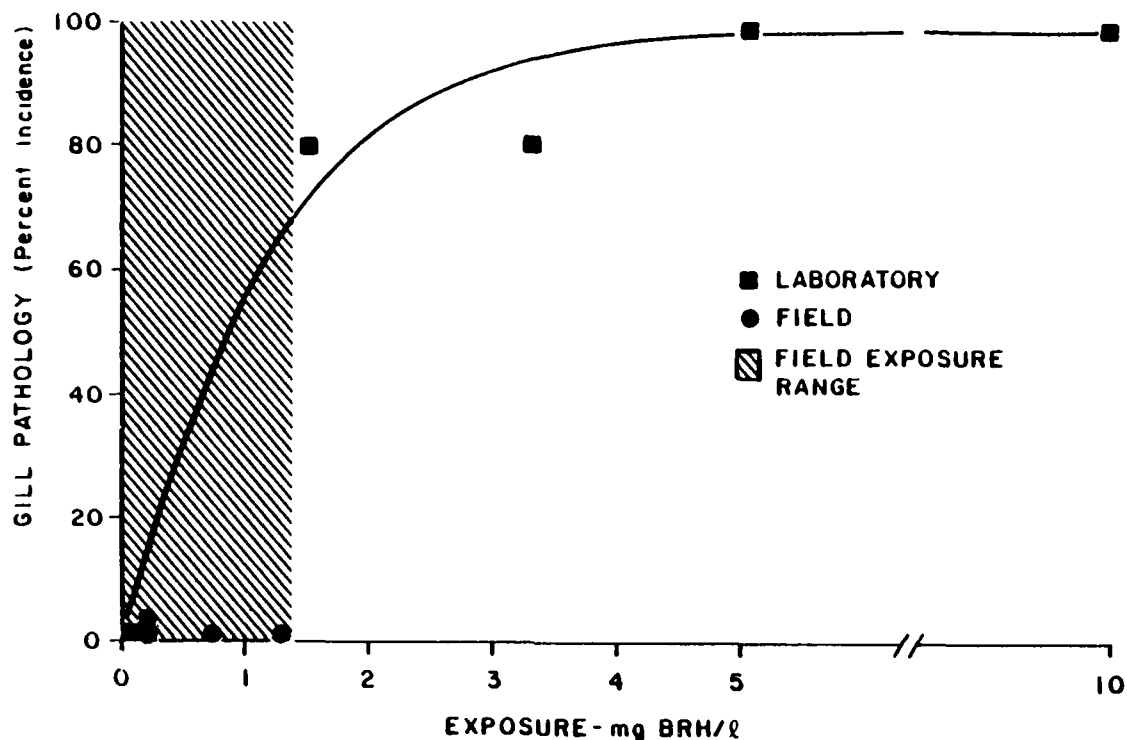


Figure 10. Percent incidence of gill pathology in *M. edulis* measured in the laboratory and field

interpretation is supported by the data presented in Figures 10 and 11, where the magnitude of the field exposure was below that required to elicit a response in the laboratory. Concurrence between laboratory and field was excellent for all other sampling periods.

68. Residue-effect comparisons. There was a direct relationship between exposure to BRH sediment and subsequent tissue residues for stable high-molecular weight compounds (i.e., PCBs) in *M. edulis*, as confirmed by the data collected during the laboratory experiments. Because of this relationship, residue concentrations of PCBs can be assumed to be indicative of exposure concentrations for BRH sediments. This relationship is particularly important in the field, where it is difficult, if not impossible, to collect direct, continuous monitoring data of exposure conditions.

69. The histopathology data for *M. edulis* exposed in the laboratory illustrate the relationship between exposure to BRH sediment and the incidence of pathology. Since there is a positive correlation between BRH sediment concentration and eight contaminant tissue residues (except for phenanthrene,

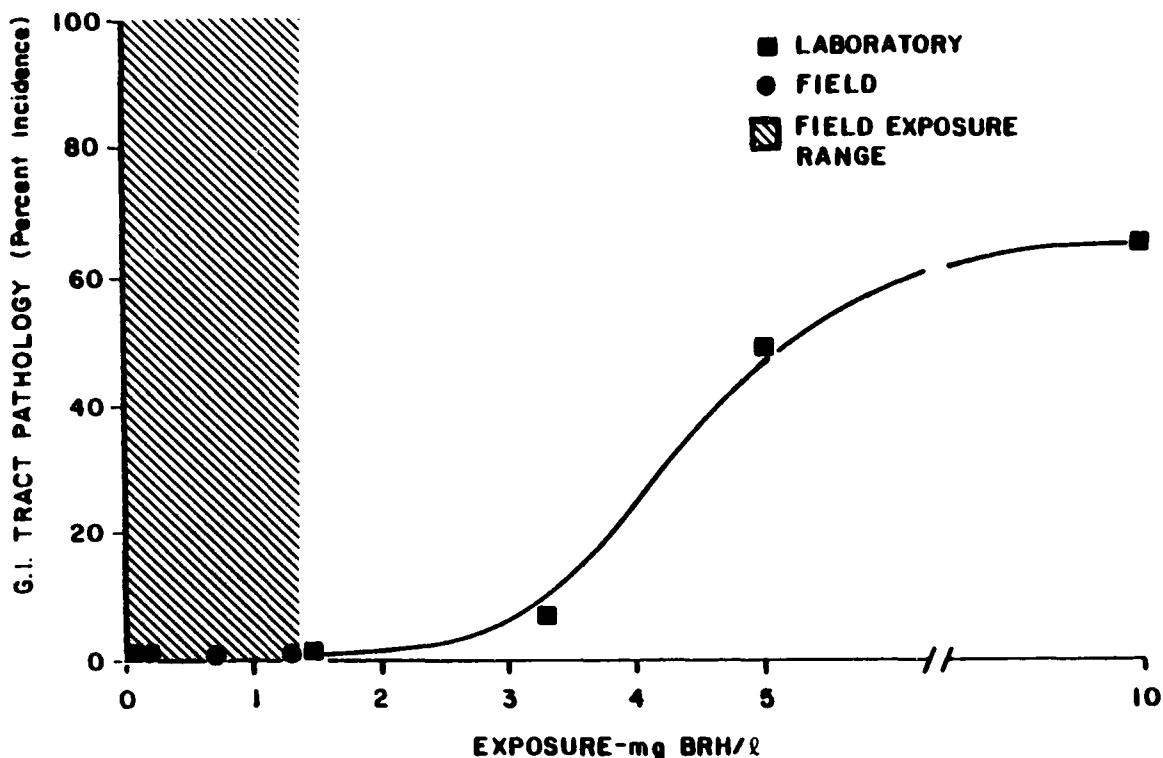


Figure 11. Percent incidence of gastrointestinal tract pathology in *M. edulis* measured in the laboratory and field

cadmium, and chromium) in *M. edulis*, it follows that observed incidences of histological changes also correlate with those contaminant-forming tissue residues. There were no residue-effects relationships determined for *M. edulis* in the field, due to the absence of observed histopathological effects.

Nephtys incisa and
Neanthes arenaceodentata

70. Laboratory documentation. Histopathological changes were noted in the epidermis and muscle of both *N. incisa* and *N. arenaceodentata*. These changes were characterized by degeneration of parapodial muscles and metaplasia of the epidermis. In *Neanthes*, histological changes were reported for the mucus-secreting cells in 100 percent of the organisms experiencing exposure to 200-mg/l suspended and 100-percent bedded BRH sediments. The secretion of mucus is essential for facilitating movement over sediment surfaces and as an adhesive for the construction of burrows. Interference with this function has important ecological implications for these species. It is

interesting to note that similar histological changes in the mucous cell system were observed in the tube-building amphipod.

71. The results of these studies lead to the following general conclusions: histological responses are not more sensitive than traditional measures of survival in short-term tests (<10 days) and are of similar sensitivity to sublethal responses in longer term tests (30 days). The value of histopathology lies more in its interpretation and explanation of the causes of mortality or other observed sublethal responses. Considering the subjective nature of this response, it is surprising to find the high degree of reproducibility evident in this study. The strength of this approach lies more in its ability to indicate long-term sublethal stress and to provide supporting evidence and explanation for organismal level responses.

72. Field verification. *Nephtys incisa* exposed to suspended BRH sediment and bedded reference sediment in the laboratory exhibited only one histological change, that involving the thickening and darkening of the parapodial epidermis. This same response was also reported in the laboratory documentation studies on BRH sediment (Yevich et al. 1986). This response appears to result from contact with BRH sediment, does not appear to be a pathological condition, and has not been treated as such in this report.

73. The exposure-response relationship for the incidence of epidermal thickening in *N. incisa* exposed in the field and laboratory is illustrated in Figure 12. The laboratory data indicate that the incidence of pathology increased with increasing exposure to BRH suspended sediment. These data indicate that the threshold for significant increase in epidermal thickening (65 percent) occurred at 200 mg BRH/l. The 18-percent increase noted at 100 mg is not considered significant. No histopathological changes were noted in field-exposed *N. incisa* at FVP sampling stations during this program (Yevich et al. 1987). This is not unexpected since the range of field exposures in Figure 12 are below the exposure threshold (200 mg/l) determined from the laboratory studies.

74. Residue-effects comparisons. The histopathological changes found in the worms appear to be of minor importance (compared to impact upon major organ systems) and occurred only in individuals exposed in the laboratory. No histopathology was noted in specimens collected at the BRH disposal site. Consequently, there is no basis for determining a relationship between tissue

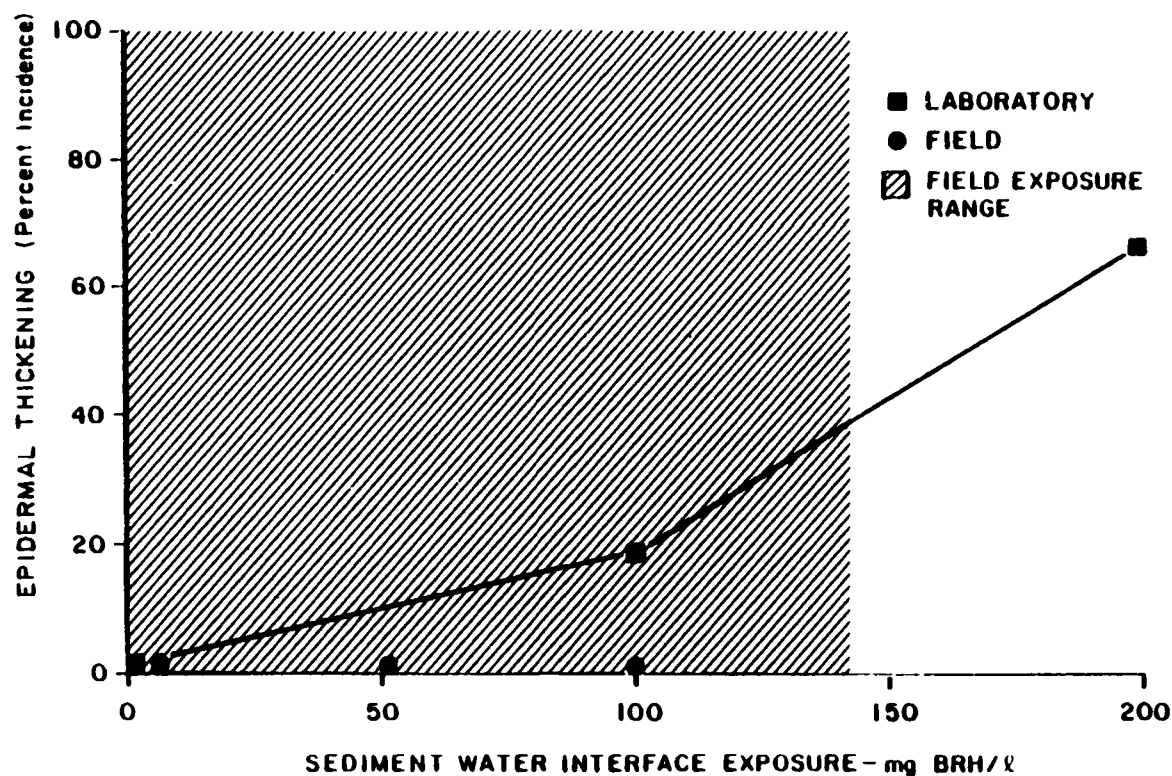


Figure 12. Exposure-response relationship for epidermal thickening in *N. incisa* in the laboratory and field

residues and changes in epidermal thickening in *N. incisa* exposed to BRH sediment.

Ampelisca abdita

75. There were well-defined patterns of histological change in *A. abdita* exposed to BRH bedded and suspended sediments. Necrosis of gill epithelium and lamellae and loss of normal gill architecture were detected in all organisms exposed to 150-mg/l suspended BRH sediments. Similarly, all animals exposed to 12-percent bedded or 50-mg/l suspended BRH sediments had atrophied and vacuolated mucous cells and mucous tube glands, as well as a loss of the basophilic staining granular material. The types of pathologies were similar whether bedded sediments or suspended sediments were the source of contamination. Pathologies were observed at exposure concentrations similar to those causing mortality and, while providing insight into the causes of mortality, did not provide an added measure of sensitivity. Reproducibility was excellent in the definitive tests due to low variability. There was no field verification component with this species for histopathology.

Yoldia limatula

76. *Yoldia limatula* showed no histopathological abnormalities in exposures of 100-percent bedded BRH sediment. The lack of both histological effects and mortalities is surprising since the species is an infaunal deposit-feeding bivalve mollusc that should have experienced maximal exposure. A possible explanation is that *Y. limatula* did not actively feed and process sediment in the high-BRH treatments and thus did not receive the expected exposures. This is supported by results of feeding observations (Rogerson, Schimmel, and Hoffman 1985) and, coupled with the short duration of the test, probably accounts for the absence of effects. There was no field verification component with this species for histopathology.

Bioaccumulation

Mytilus edulis

77. Laboratory documentation. The bioaccumulation potential of available contaminants within the dredged material was assessed by: (a) determining the qualitative and quantitative aspects of the bioavailable contaminants within BRH dredged material which are accumulated and (b) examining the uptake and depuration kinetics of the major contaminants in *M. edulis* and *Nereis virens*. Initially, *M. edulis* showed a rapid uptake of PAH and PCB compounds, followed by a slight decrease in the residue concentrations of most compounds during the next 2 weeks and then an increase, with the highest concentrations found at day 28. The PCB compounds with molecular weights above C_{17} PCB were not effectively accumulated by the mussels; similarly, PAHs of higher molecular weight were not accumulated to the same extent as lower molecular weight PAH compounds. Application of a nonlinear model to the mussel data indicated that steady-state concentrations of PCBs had been reached during the 28-day exposure period. From the shapes of the uptake curves for the PAH compounds, it appeared that steady-state concentrations of PAHs also had been reached during the 28-day exposure (Lake, Hoffman, and Schimmel 1985). Uptake of metals from BRH suspended sediments by *M. edulis* included iron, copper, chromium, zinc, lead, cadmium, and arsenic. The mean tissue concentrations of these metals were significantly different from the respective mean concentrations in control mussels.

78. Field verification. Research in this component of the FVP was designed to compare bioaccumulation of contaminants in the laboratory studies with bioaccumulation found in field organisms. The approach was to identify contaminants in organisms from laboratory studies and from field exposures and, where possible, to compare residue concentrations and exposures from both locations. While small differences in the patterns of accumulated PCBs were noticed in the initial phase of the laboratory bioaccumulation studies, the PCB distributions and patterns in both *M. edulis* and *N. incisa* at steady state were quite similar regardless of the mode of exposure. The similarities of PCB distributions and patterns suggest that for some neutral organic compounds, predictions of the maximum attainable concentrations of contaminants in organisms from a specific dredged material may be made by simply measuring the organic carbon content of the material, its contaminant concentrations, and the lipid concentrations of the target organism(s). This view, referred to as equilibrium partitioning, considers the bioaccumulation of neutral organic contaminants, such as PCBs, to be part of a redistribution of contaminants between the organic carbon of the sediments (the source) and the lipids of the organisms (the sink) (MacKay and Patterson 1981; McFarland 1984; Karickhoff and Morris 1986; McFarland and Clarke 1986; Lake, Rubinstein, and Pavignano 1986; US Army Corps of Engineers 1987).

79. The exposure-residue relationship for PCB in *M. edulis* for laboratory and field exposure is illustrated in Figure 13. PCBs were chosen to examine the quantitative aspects of field verification because of their persistence, equilibrium-partitioning, and kinetics. The uptake and resultant PCB tissue residues, when corrected for lipid content, increased proportionally with increasing BRH suspended sediment exposure in the laboratory. PCB tissue residues from *M. edulis*, deployed immediately after disposal, agreed well with estimates from the laboratory exposure-residue relationship. Thus, field PCB tissue residues could be accurately predicted from laboratory exposure-residue relationships and field exposure information.

Nephtys incisa and *Nereis virens*

80. Laboratory documentation. The exposure of worms *N. virens* to BRH sediment resulted in accumulation of organic compounds, PCBs and PAHs, and the metals copper and chromium. The PCBs accumulated by the worms showed a pattern similar to that observed in the sediment and showed no apparent decreases

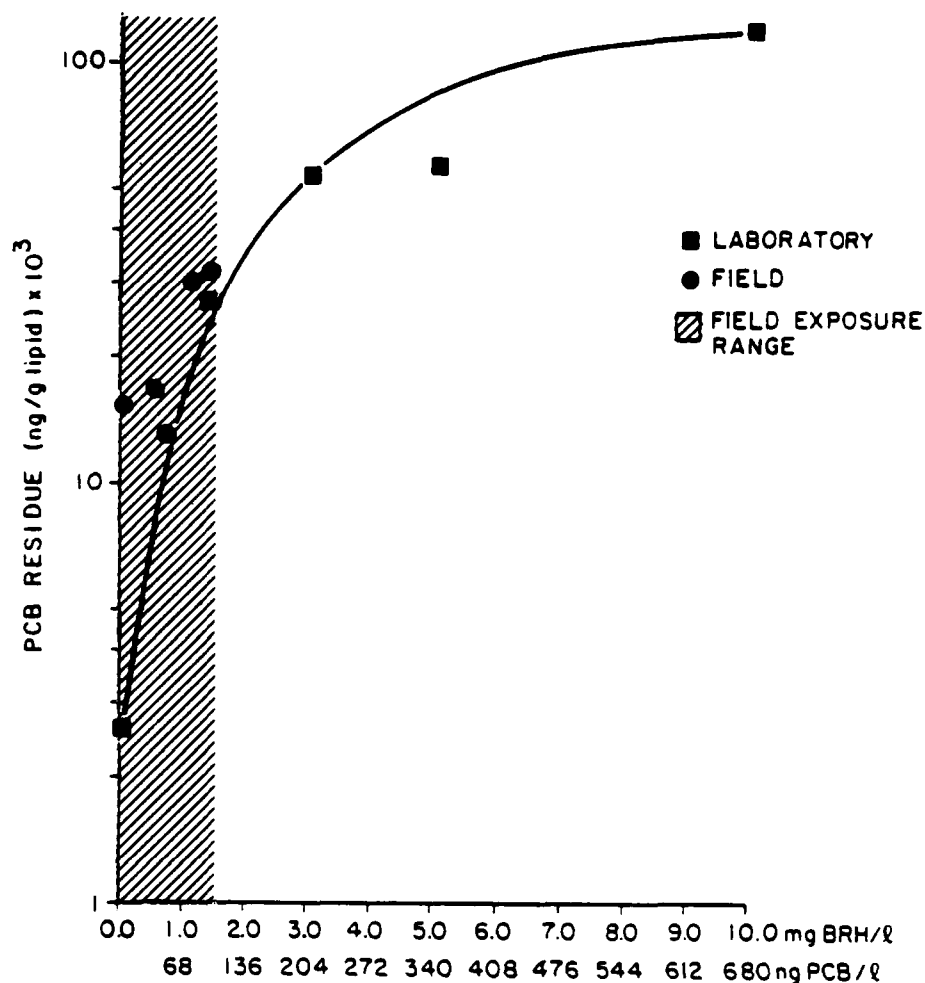


Figure 13. Field verification of tissue residues (PCB) in *M. edulis*

in total concentrations over the depuration period. Exposed worms accumulated PAHs to concentrations that were orders of magnitude greater than those in the reference worms but, in contrast to the PCBs, the concentrations of PAHs in the worms fell rapidly during depuration.

81. Field verification. The exposure-residue relationship for PCB in *N. incisa* in the laboratory was constructed from data from both bedded and suspended BRH sediment exposures (Figure 14). Results indicate that PCB residues increased predictably with increasing exposure to BRH sediment. Field PCB tissue residues and their corresponding estimated exposures coincide with laboratory data. Thus, for *N. incisa*, the laboratory exposure-residue

relationship could be used in conjunction with field exposure to accurately predict field PCB tissue residues.

82. Other neutral compounds, such as PAHs, have a greater range of physical-chemical properties than PCBs, and the former are metabolized. This results in their being more dynamic in bioaccumulation studies. Therefore, they were not used to examine the issue of field verification. Considering the differences in PAH properties, sources, and availability, it is not surprising to find irregularities in their accumulation curves for laboratory exposure studies. Likewise, these properties are probably reflected in differences in the patterns of PAHs accumulated in organisms from the laboratory and field exposures.

83. Exposure to organic contaminants, such as PCBs, in dissolved form, on suspended particulate matter (mussels), and in pore water and sediments (worms) can be simplified by assuming that the total exposure concentration of PCBs resides on the particles or in the sediments. When this is done, the calculated bioaccumulation factors (BAFs) for mussels and worms are quite similar. Similar calculations with the more reactive and possibly less available PAHs (due to incorporation in soot particles) show greater differences.

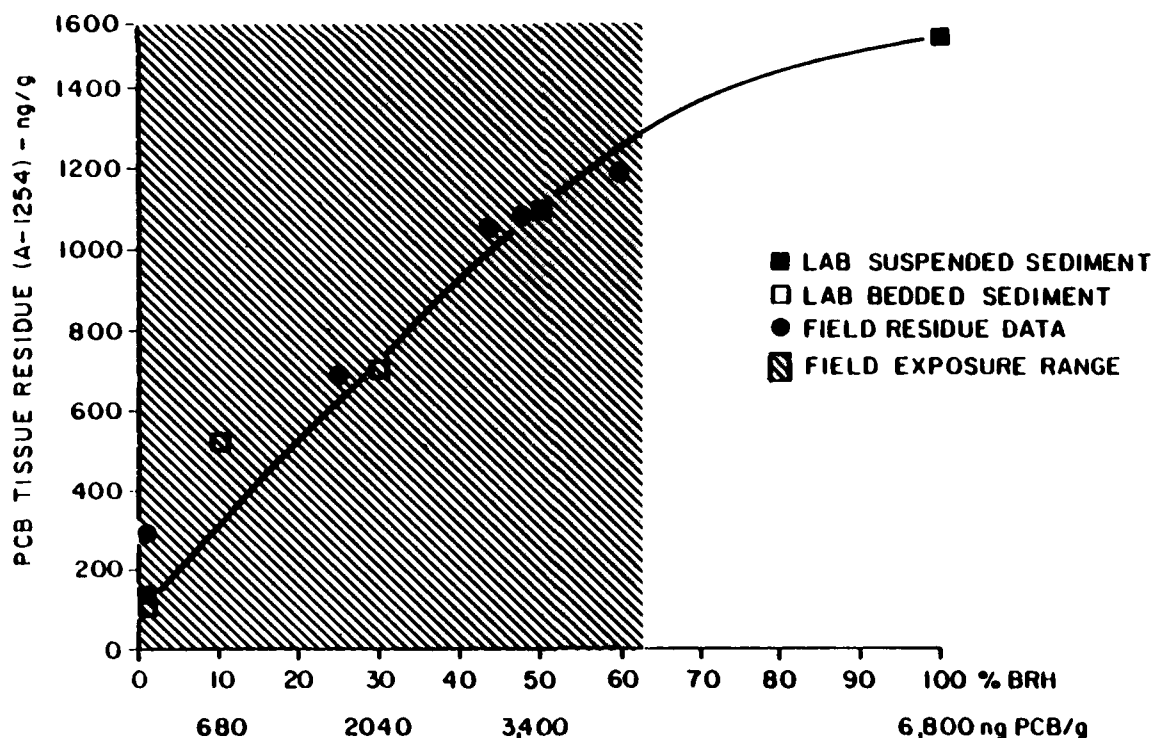


Figure 14. Field verification of tissue residues (PCB) in *N. incisa*

Similarities in worm and mussel BAFs suggest that modeling bioaccumulation of some organics (at least less reactive compounds such as PCBs) as a partitioning of contaminants between organisms (worms and mussels) and sediment or suspended sediment shows promise as a predictive technique for assessing the accumulation of organic contaminants from dredged material and other mixed wastes.

Population Response Parameters

Mysidopsis bahia

84. Laboratory documentation. *Mysidopsis bahia* was used in this program as a surrogate species for studying chronic and population responses. Field verification of laboratory responses was not possible because this species was not indigenous to the disposal site and the biology of a local congeneric species was not known well enough. The responses measured in *M. bahia* included acute and chronic mortality, growth, and reproduction, which were used in demographic models to estimate the intrinsic rate of population growth. Laboratory exposures were conducted with proportions of BRH and REF sediments mixed to create varying contaminant concentrations that flowed continuously over bedded REF sediment for the 28- to 36-day test duration.

85. The results from acute and chronic toxicity tests using BRH sediments (anaerobic prior to their introduction into the exposure system) are summarized in Table 4. The 96-hr LC50 to suspended BRH sediments was 290 mg BRH/l. Growth was not significantly affected at BRH exposures ≤ 156 mg/l. Production, estimated from the number of young per available female reproductive day, had an EC50 value of 42 mg/l of BRH suspended sediment. The intrinsic rate of population growth was calculated from life tables that utilized age-specific survival and reproductive data from life cycle tests (Gentile et al. 1985). The EC50 value for population growth rate was 42 mg/l of BRH sediment. These studies demonstrate the successful application of long-term chronic test methods using suspended BRH sediment exposure with *M. bahia*. Growth, reproduction, and population parameters measured using this method responded in a consistent and reproducible manner.

86. Field verification. The field verification portion of this study employed the same experimental design as in laboratory documentation except that the BRH sediment was oxidized prior to introduction into the exposure system. These tests were not designed to determine reproducibility of the

Table 4
Comparative Acute and Chronic* Toxicity of Reduced and Oxidized BRH
Sediment (milligrams/litre) to *M. bahia* and *A. abdita*

Endpoint	<i>M. bahia</i>		<i>A. abdita</i>	
	Reduced	Oxidized	Reduced	Oxidized
96-hr LC50	290	262	91	80
Growth	>156	25	2.3	2.2
Chronic mortality	150	100	12.5	11.0
Reproduction	42	18	4.5	2.2
Intrinsic rate of growth, r	42	8.0	4.5	1.1

* Chronic values are concentrations of BRH sediment resulting in significant (P = 0.05) response difference from controls.

responses but rather to estimate the response threshold. The 96-hr LC50 value was 262 mg/l. The chronic responses studied were growth, reproduction, and intrinsic rate of population growth. Statistically significant differences in growth were measured at 25 mg/l, while the percentage of females with eggs was significantly reduced at exposures of 21 mg/l. The percentage of females with eggs proved to be an excellent predictor of productivity where statistically significant effects were recorded at exposures of ≥ 21 mg/l. The EC50 value for productivity occurred at 18 mg/l BRH sediment. Finally, the intrinsic rate of population growth calculated from survival and reproductive data indicated that zero population growth would occur at 10 mg/l and strongly negative growth rates (thus, rapid population extinction) at 21 and 25 mg/l.

87. In general, the acute toxicities of aerobic and anaerobic BRH sediments were not significantly different. Chronic responses, however, were significantly more affected by exposure to aerobic sediments. In particular, growth decreased significantly at 25 mg/l of aerobic sediment but was unaffected even at 156 mg/l of anaerobic sediment. Reproductive response thresholds decreased from 42 to 18 mg/l, while the intrinsic rates of growth response threshold decreased from 42 to 18 mg/l for anaerobic and aerobic sediment exposures, respectively (Table 2).

Ampelisca abdita

88. Laboratory documentation. *Ampelisca abdita* is an infaunal tubiculous amphipod that inhabits surficial sediments and is indigenous to the disposal site. The biological responses studied in *A. abdita* were acute and chronic mortality, growth, fecundity, and population growth rate. The exposure design consisted of proportions of BRH and REF suspended sediments flowing continuously over bedded REF sediment for up to 56 days. The 96-hr LC50 values of 84 and 91 mg/l determined from replicate anaerobic suspended sediment exposures were not significantly different (Gentile et al. 1985). Growth in male *A. abdita* was not significantly affected after 28 to 32 days exposure to 4.5 mg BRH/l, while similarly exposed females were significantly smaller than the males at 4.5 mg BRH/l.

89. Fecundity, measured as total young produced, was dramatically reduced by BRH suspended exposures >2.3 mg/l. This was the result of decreased female growth, which caused smaller adult females. Mills (1967) has shown that the number of eggs per female in *A. abdita* is directly related to female size. While the eggs:female ratio decreased at the 5.0 mg/l exposure, when adjusted for female size by covariance analysis, apparent treatment differences disappeared. Therefore, impairment of fecundity in *A. abdita* resulted from reduced female growth due to BRH sediment exposure (Gentile et al. 1985).

90. Intrinsic rates of population growth were determined from survival and fecundity data in replicate experiments. The effects of impaired growth resulting in delayed maturation were evident in the intrinsic rates of growth. In both experiments, a distinct exposure-response relationship was demonstrated from which EC50 values of 2.2 and 4.5 mg/l were estimated. Survival, growth, fecundity, and intrinsic rate of population growth in *A. abdita* showed consistent and reproducible responses when applied to evaluating the effects of dredged material suspended solids.

91. Field verification. The field verification phase of this study utilized an experimental design similar to that described above, except that aerobic rather than anaerobic sediments were introduced into the exposure systems. Direct field verification of laboratory responses was not possible since *A. abdita* did not recolonize the disposal site in large numbers, and its distribution around the site was not spatially or temporally consistent. The 96-hr LC50 was 80 mg/l, and the 10-day LC50 was 12 mg/l for aerobic suspended

BRH sediment. The growth rates of female *A. abdita* were significantly reduced after 28-day exposure to 2.0 mg/l. Growth patterns were similar to those described above, where effects occurred at 5.0 mg/l with anaerobic sediments.

92. Fecundity, measured as the number of eggs per female, was correlated with female size and was significantly reduced at an exposure of 2.2 mg/l BRH suspended sediment. The ovigerous females, in addition to being smaller and thus producing fewer eggs, also had lower fecundities when compared to similar-sized animals in the REF and 1.1-mg/l treatments. This is supported by examining the number of young produced. The total number of young produced in the REF treatment was over eight times that in the 2.2-mg/l BRH treatment. What was unexpected was that the productivity in the 1.1-mg/l treatment also was reduced significantly, even though fecundity (eggs/female) was similar to the REF sediment control. A possible explanation for this is that either hatching success or juvenile survival was affected, neither of which was directly measured in this study. In addition, the mean size of juveniles was significantly larger in the REF controls than in the 1.1- and 2.2-mg/l BRH sediment treatments. The REF juveniles were probably released sooner and were growing faster than juveniles in the BRH treatments. If so, they would mature faster and reproduce sooner than juveniles in BRH treatments. This would compound the effects at the population response level since time to sexual maturation and first reproduction have a major impact on the intrinsic rate of population growth. In fact, population growth rates were decreased 65 and 75 percent, respectively, at 1.1 and 2.2 mg BRH/l, which resulted in an estimated population growth EC50 of 0.6 mg/l BRH suspended sediment.

Benthic Recolonization and Community Structure

93. The objectives of this portion of the study were to describe the effects of the disposal of dredged Black Rock Harbor sediments on the predisposal community and to describe the mode and pattern of recolonization of the FVP site. The recolonization process was measured by documenting the rate of recolonization of the FVP site and comparing this with the ambient (control) community. The parameters used to describe recolonization and convergence with the predisposal system were: species numbers, abundances of numerically dominant species, degree of infaunalization (successional stage),

and depth of biogenic mixing of the bottom sediments (another measure of infaunalization). The latter two parameters were described using the REMOTS interface camera (Rhoads and Germano 1982).

94. Recovery of a disturbed habitat can be defined as a return to background or ambient conditions. These ambient conditions could be based on those present at a site prior to the disturbance or those relative to a reference site, or some combination of both. The predisposal surveys at the FVP site were conducted to examine the spatial heterogeneity of the site and to assess ambient predisposal conditions relative to the reference site (REF).

95. The predisposal benthic community at the FVP site was characteristic of the soft-bottom, nonperturbed system in Long Island Sound. This *Nephtys-Nucula* community (Sanders 1956) occurs where organic concentrations are between 1 and 10 mg carbon/gram of sediment, with most of the grain sizes falling within the silt-clay range. Typically, on a small scale (within-station), these species abundances are distributed in patches, displaying a mosaic species distribution. Replicate station grabs contained almost all the species but had different population abundances of individual taxa. On a larger scale (i.e., the entire disposal area), these population mosaics were consistent over the whole sampled site. This community structure and its component species appear to be well adapted to silty sediments and are characteristic of similar biofacies described for Long Island Sound (Sanders 1956), Narragansett Bay (Phelps 1958), and Buzzards Bay (Sanders 1958).

96. Few differences were detected between FVP stations prior to the disposal operation, both for the quantitative grab samples and REMOTS parameters (Scott et al. 1987). Where station-to-station differences were detected, they appeared to be local anomalies. No large-scale disturbance gradients were apparent in the baseline data. One exception to this general observation is that the REF station exhibited a seasonal pattern of significant differences in some species abundances prior to the disposal operation. The species composition, rank abundances, and REMOTS parameters showed the REF station to be qualitatively very similar to the disposal site. Given that the trends in seasonal and yearly abundances were similar at both the FVP site and REF station, the REF site appeared to be appropriate for making comparisons with postdisposal changes measured at the FVP site.

97. The benthic community, sampled at the FVP site prior to disposal (baseline) and off the disposal mound following disposal (until December

1983), was dominated by a subsurface infaunal deposit-feeding assemblage consisting of the protobranch bivalves *N. annulata* and *Y. limatula* and the polychaete worm *N. incisa*. All three of these organisms are stage III taxa (sensu Rhoads and Germano 1982) that have mean life spans greatly exceeding 1 year. Reproduction may take place two or more times a year. These organisms are important in bioturbating the sediment column, and this biogenic mixing controls both pore water and solid-phase chemistry. This subsurface deposit-feeding assemblage was overlain by near-surface populations of the deposit-feeding polychaete *Mediomastus ambiseta* and the suspension-feeding matrid bivalve *Mulinia lateralis*. These two species are well-known members of stage I series representing opportunistic adaptive strategies. Mean life spans are less than 1 year, and reproduction of the population occurs several times per year. The sympatric association of opportunistic colonizers (stage I taxa) with longer lived species (stage III taxa) is common in estuaries and embayments. These early colonizers are typically very abundant on disturbed bottoms. This assemblage type has been previously described for the CLIS silt-clay facies (Sanders 1956, Michael 1975) and for the silt-clay basinal facies of Buzzards Bay (Sanders 1956).

98. The temporal pattern of recolonization consisted of two separate processes operating at different time scales. The first process was the immediate recolonization of the dredged material mound, which occurred during the first 6 months following disposal. Short-lived, early colonizing species (*Mulinia*) populated the mound in significant densities, and in some cases were most abundant at the CNTR station. This phase of the recolonization of the FVP site was not unlike that seen for other disturbed sites within Long Island Sound and elsewhere (Rhoads, Aller, and Goldhaber 1977). The greater abundances at the CNTR station are not surprising since many early colonizers thrive in disturbed or defaunated habitats (McCall 1977) where competition for space or the biologically mediated geochemical conditions of the sediment do not pose problems for recruitment.

99. The second component of the recovery process, which may begin concurrently with the initial colonization, is the progressive development of subsurface bioturbation associated with reestablishment of the long-lived species. The time scale of this process may be on the order of 1 to 2 years or more. It was not until 19 months after disposal that head-down feeding voids were observed on REMOTS images at the FVP site mound stations, even

though the major frequency mode of the biological mixing depth (BMD) at those stations had converged with that of REF within 1 year. The CNTR station continued to have a significantly lower BMD, even though head-down deposit feeders from the ambient community were among the recolonizers. *Nephtys incisa* abundances gradually declined from May 1982 (predisposal) through December 1983 (postdisposal) at all stations. There was some recruitment at CNTR in December 1983. *Nephtys incisa* densities remained low at both REF and CNTR stations in June of 1984 and 1985, with significant recruitment occurring between June 1985 and October 1985 following Hurricane Gloria. *Yoldia* was recruited in equal densities at both the REF and CNTR sites between September and December 1983, and the stations showed similar population patterns throughout the study except that the *Yoldia* recruitment and subsequent density decreases following Hurricane Gloria were greater at the REF station. The recolonization pattern for *Yoldia* and *Nephtys* showed that the CNTR station was recovering and converging with the REF station. The protobranch *Nucula*, however, did not recolonize the mound in significant numbers, which is likely to be related to the significant sand fraction at CNTR. *Nucula* is also absent from other sand-covered disposal mounds in CLIS.

100. The failure of the ambient stage III assemblage (*Nephtys*, *Nucula*, and *Yoldia*) to become established at the CNTR station by June 1985 may have been due to grain-size effects since other deep bioturbating organisms were present, although in low densities (Figure 15). It may be that the sand lens on the surface of the mound was effectively capping the subsurface contaminated BRH sediments. As a result, when the head-down feeders grew to a size where feeding depths penetrated the subsurface contaminated silts, feeding activities and survival may have been impaired.

101. Hurricane Gloria had a major impact on the recovery process at the FVP site. Cluster analyses using dominant species abundances and REMOTS parameters, together with numbers of species and individuals, showed that the CNTR and REF stations were virtually indistinguishable after Hurricane Gloria in October 1985. The mean number of species per quadrat was similar at these two stations, although the high numbers of individuals indicated that the mound was undergoing another colonization phase similar to that documented in December 1983 (Figure 16). The retrograde conditions in the BMD and Organism Sediment Index (OSI) (Scott et al. 1987) at the REF station also indicated a disturbed bottom (Figure 15). This resulted from the storm-induced sediment

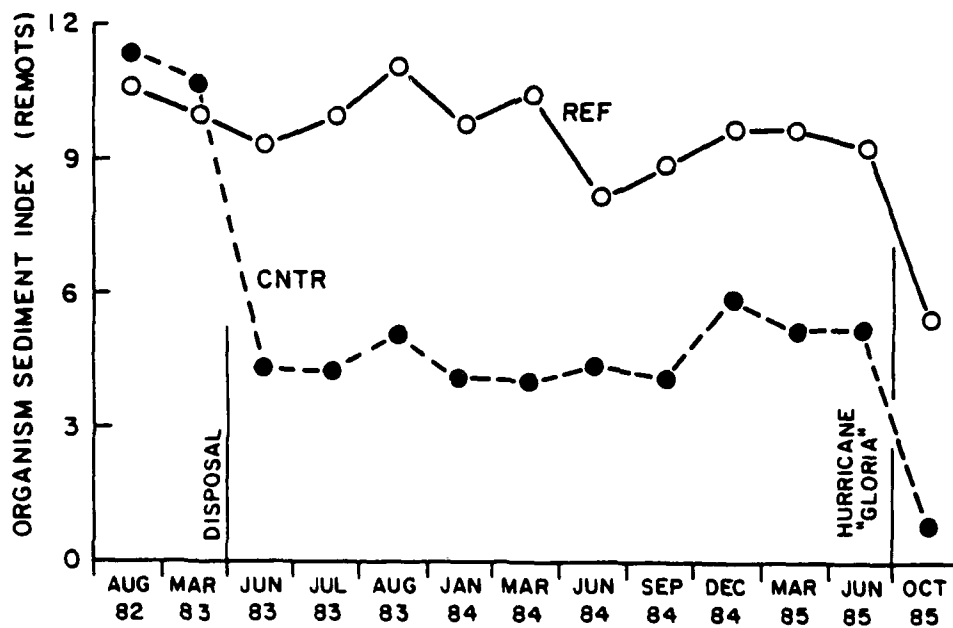
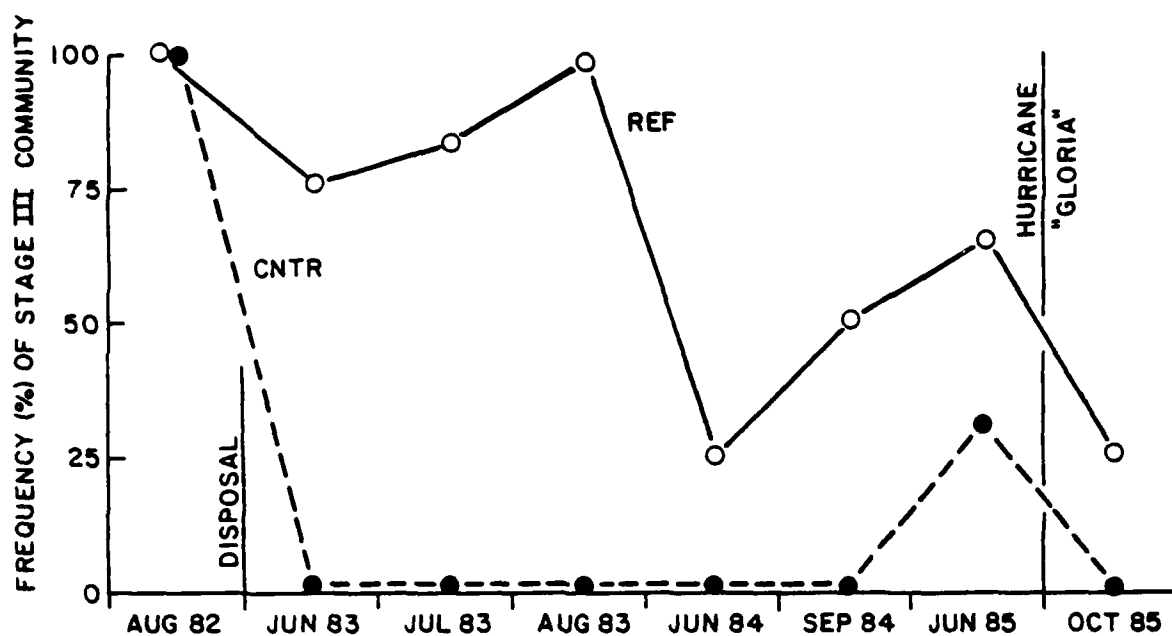


Figure 15. Temporal and spatial patterns of the community parameters, successional stage, and organism sediment index at the FVP disposal site

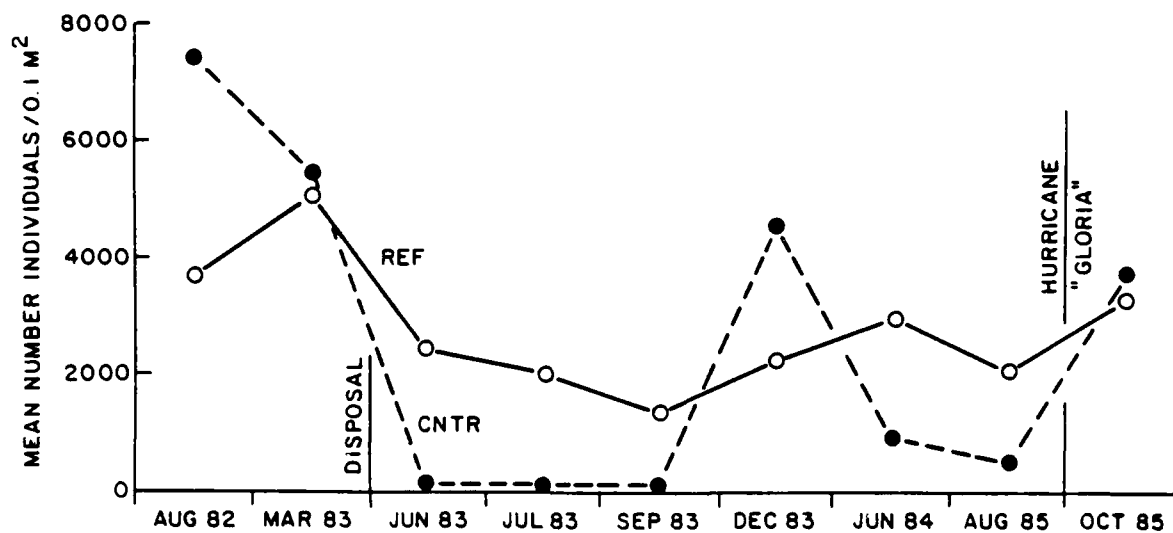
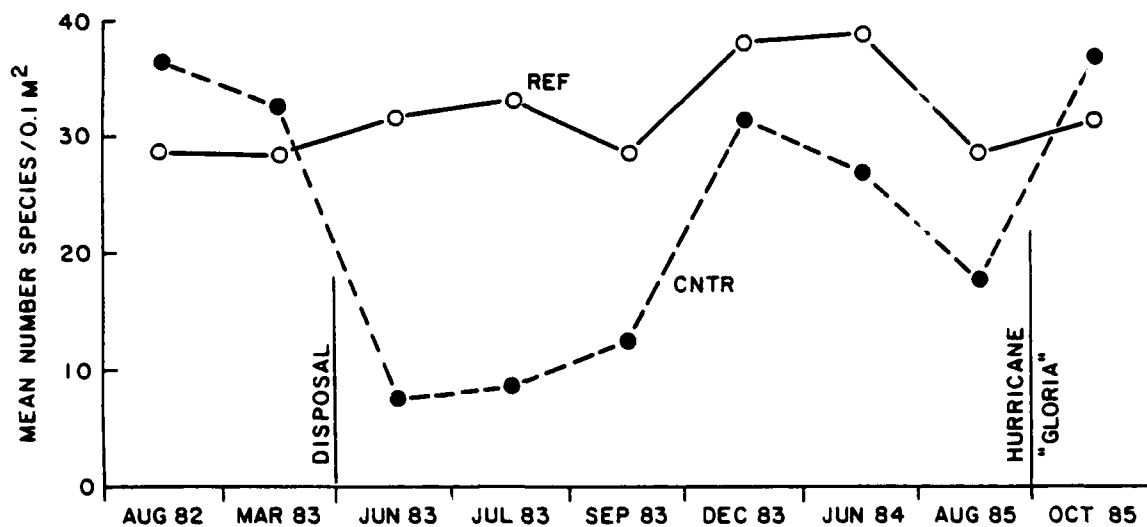


Figure 16. Temporal and spatial patterns of the mean number of species per quadrat and mean density of all individual infaunal organisms at the FVP disposal site

entrainment, which removed 2 to 4 cm of surface sediment in this part of Long Island Sound. In addition, the commonness of species composition in October 1985 indicated that extensive recolonization was occurring. Passive dispersal of adult organisms from the ambient seafloor to the mound may have also been involved in this process.

PART III: DISCUSSION

102. The results of the laboratory documentation, field verification, and residue-effects components of this program lead to a series of conclusions regarding: the sensitivity of species and biological responses, the reproducibility of exposure-response relationships, the degree to which laboratory responses can be field verified, the correlation between contaminant tissue residues and biological effects, and the utility of the test methods for evaluating dredged material impacts.

Species and Response Sensitivity

103. Of the several organisms examined during the FVP, only the filter-feeding bivalve mollusc *M. edulis* and the infaunal deposit-feeding polychaete *N. incisa* were used to address all three objectives of this program. The infaunal crustacean *A. abdita* and the epibenthic crustacean *M. bahia* were not used for studying field verification or residue-effects relationships but were used for evaluating chronic responses, including population responses.

Between the two crustaceans, where the same suite of responses was measured, *A. abdita* was consistently more sensitive than *M. bahia* by a factor of 3 to 4 for acute responses and by a factor of 10 chronic responses (Table 4). Comparing similar responses between *M. edulis* and *N. incisa* indicated that the former was 5 to 10 times more sensitive than the latter to BRH suspended sediments. The response common to all species was growth, which displayed the following ranking of sensitivities: *A. abdita* = *M. edulis* > *M. bahia* = *N. incisa* (Gentile et al. 1987). Therefore, *A. abdita* and *M. edulis* have comparable sensitivities to BRH sediment and are considerably more sensitive than *M. bahia* and *N. incisa*.

104. The ranking responses of the crustaceans from least to most sensitive is as follows: acute mortality, chronic mortality, growth, fecundity or reproduction, and intrinsic rate of population growth. A similar ranking among the responses in *M. edulis* and *N. incisa* is illustrated in Table 5. Comparing responses between species indicates that scope for growth and growth in *M. edulis* were of comparable sensitivity to the growth, reproduction, and population responses in *A. abdita* (Table 4).

Table 5
Ranking of Response Sensitivity in *M. edulis* and *N. incisa*
to BRH Sediment Exposure (milligrams/litre)

Response	Species	
	<i>M. edulis</i>	<i>N. incisa</i>
Growth	1.5*	100
Scope for growth	1.5	NA
Bioenergetics	NA	100
Sister chromatid exchange	NA	NA
Histopathology	1.5	200
Adenine nucleotide pool	>5.0	>200
Adenylate energy charge	>10.0	>200

* Tabular values represent lowest concentration causing a change in response.

Biological Response Summary and Evaluation

Bioaccumulation

105. The laboratory methods that were developed for exposure of *M. edulis* to suspended sediments were found to maintain constant concentrations of total suspended sediment and the proper ratio of contaminated dredged material and reference sediment. The resulting contaminant uptake patterns in *M. edulis* were directly related to BRH suspended sediment concentrations. The tissue residue values for PCBs in *M. edulis*, when corrected for organism lipid content, were linearly related to contaminant concentration. PCB and PAH tissue residues reached steady state for PCBs and PAHs within 28 days for *M. edulis*. Similarly, laboratory methods were developed to expose the infaunal polychaete *N. incisa* to contaminated dredged material through both suspended and bedded sediment exposures. Tissue residues in both exposures increased with increased exposure to BRH sediment. After 55 days exposure to contaminated bedded sediments, *N. incisa* PAH tissue residues reached apparent steady state, but PCB tissue residues did not. After 42 days of exposure to contaminated suspended sediment, both PAH and PCB tissue residues reached an apparent steady state.

106. Evaluation of the utility of compounds for examining the relationship between tissue residues in the laboratory and field with *M. edulis* and *N. incisa* showed that, in general, PCBs were the most useful compounds. Further research is needed on the equilibrium partitioning approach for estimating the maximum concentration of some neutral organic contaminants (such as PCBs) that can be bioaccumulated from a specific dredged material. This approach was examined only cursorily in the FVP program. The similarities of the distribution and patterns of PCBs in organisms from the laboratory and field exposures in this study suggest that similar kinetic processes govern these distributions. The development of a simple test to estimate the maximum residue concentrations would be of great assistance for facilitating disposal decisions. Careful consideration must be given to the organism(s) selected for laboratory bioaccumulation tests. This consideration should include the available knowledge on feeding modes and habitat utilization so that exposure zones can be established and exposure-residue relationships can be determined. Further, the spawning habits and associated changes in lipid concentrations of the organisms must be accounted for in the experimental design, since these factors are known to affect bioaccumulation. Finally, the organism's ability to metabolize contaminants of interest should be considered.

Scope for growth

107. The results of the laboratory study indicated that the mussel residue concentrations were indicative of exposure conditions and that the SFG index was useful for measuring the subsequent biological effects of those exposures. An inverse relationship was observed in the laboratory between SFG and BRH exposure concentration. Likewise, SFG was inversely related to the tissue residue concentrations of some of the contaminants present in the dredged material. The lower SFG values observed in BRH-exposed mussels were attributable to reduced clearance rates observed in the laboratory. In addition, mussels with lower SFG values exhibited reduced growth rates. A qualitative comparison between the SFG of mussels after laboratory and field exposures indicated that the estimated BRH concentration that affected SFG in the field was similar to the range predicted from the laboratory experiments.

108. SFG measured in *M. edulis* was a sensitive, exposure-related chronic response, was highly reproducible, and correlated well with shell growth. The field verification of this response was excellent. SFG is recommended as a routine test in the predisposal evaluation processes and as a

field assessment or monitoring method for permit compliance during and immediately after disposal because biological impacts can be measured simultaneously with tissue residues.

Bioenergetics

109. The bioenergetics responses measured in *N. incisa* included growth, respiration, excretion, cumulative energy for production, and net growth efficiency. Each response was directly related to BRH exposure and was highly reproducible. Of this suite of responses, growth, production, and net growth efficiency were the most sensitive determined for *N. incisa*. Field verification, limited to excretion and respiration, correlated well with previous laboratory studies on this organism. Further development of this technique for both lab and field applications is recommended, but it is not recommended for routine use in the regulatory evaluation process.

Adenylate energy charge

110. The biological responses evaluated included the adenine nucleotide measures of ATP, ADP, AMP, adenine nucleotide pool, and AEC. These responses were measured in *M. edulis* and *N. incisa* exposed to BRH sediment in the laboratory and the field. The only significant laboratory response was a reduction in total adenine nucleotide pool concentration measured in *M. edulis* at BRH exposure concentrations higher than any estimated exposures in the field. The only field responses of note were station-related trends in all adenylate nucleotide concentrations measured in *N. incisa* 16 weeks postdisposal. However, the significance of these trends is relatively minor when viewed within the context of the total study.

111. Based upon these results, adenine nucleotide responses including AEC were insensitive to BRH suspended and bedded sediment exposures in *M. edulis* and *N. incisa* under a wide range of exposure conditions in both the laboratory and field. Consequently, they are not recommended for evaluating dredged material impacts.

Histopathology

112. The histopathological response-exposure relationships were difficult to quantify in a reproducible manner even under laboratory conditions. In *M. edulis* the percent incidence of pathology within a specific organ system was related to exposure intensity. In *N. incisa*, the only histological response detected in replicated laboratory experiments was thickening of the epidermis. However, no effects were observed in either species in the field.

This was unexpected since one of the apparent advantages of this technique was thought to be its usefulness for the field assessment of chronic impacts. However, histopathology was useful in providing insight into the causes (destruction of gill architecture) for the observed effects on scope for growth in *M. edulis*. Based upon these observations, the use of histological responses within the predisposal evaluation processes is not recommended. It may have utility in the field assessment of postdisposal impacts if the duration of exposure is adequate to allow for the induction and manifestation of the responses.

Sister chromatid exchange

113. The current ocean disposal regulations have identified the need for genotoxicity test methods in the regulatory decision-making process. The application of SCE response to evaluating the genotoxicity of dredged material has pointed out the need for additional research in this area. There was an inconsistency in the SCE response among the laboratory replicate tests, which suggests that the application of SCE to *N. incisa* needs further development before it can be recommended for routine use. The increased frequency of SCE in *N. incisa* is consistent with responses of the Ames test to extracts of BRH sediment. Whole extracts of BRH sediment elicited a clear dose-response with *Salmonella* strain TA100 in the presence of rat liver S-9. Based upon current understanding of mutagenesis, the positive results with the Ames test and the SCE response in *N. incisa* suggest a tumorigenic potential for BRH sediment. This is supported by Gardner et al. (1986), who report tumor development in oysters (*Crassostrea virginica*) and winter flounder (*Pseudopleuronectes americanus*) exposed to BRH sediment under laboratory and field conditions. The general applicability of the Ames test has been demonstrated as a prescreen for determination of mutagenic and potential carcinogenic hazards of pure chemicals, complex environmental mixtures (such as sediments), and commercial products. Because of the technical issues with the SCE procedure applied to *N. incisa*, this method cannot be recommended at this time. However, a prescreen for genotoxicity is considered to be an essential component in the evaluation of dredged material, and the Ames test can be used while development on other test methods continues.

Population growth rates

114. Acute and chronic toxicity responses, including population growth rates, were exposure-related in a highly reproducible and predictable manner in both *M. bahia* and *A. abdita*. Of the responses measured, growth, reproduction, and intrinsic rates of population growth were consistently the most sensitive responses within a species as well as between these two species. The test methods and responses developed for these species were useful indicators of sediment toxicity and are recommended for inclusion in the predisposal evaluation of dredged material.

Community responses

115. Community responses, measured as recolonization, were examined in the field to serve as a biological benchmark for this study. Enumeration of species type and calculation of population densities from sieved Smith-McIntyre benthic grab samples were complemented with a remote, rapid photographic method (REMOTS). Both methods conveyed the same general patterns of spatial and temporal impact and rates of recovery. The degree of resolution regarding community structure was, of course, quite different. The REMOTS method offered several advantages as a rapid screening tool for assessing the spatial distribution of the dredged material, for insight into the oxidation state of the seabed, and for determining stage of recolonization. It is recommended for use within this context and to complement the more detailed characterization of the infaunal community structure by species enumeration. Because the algorithm for characterizing the community structure or the OSI using REMOTS is only qualitatively described, it cannot be used to replace species enumeration at this time. Rapid reconnaissance of the benthos, such as the REMOTS method, is recommended for predisposal characterization of a disposal site and for postdisposal reconnaissance. Species enumeration methods need be used only in definitive studies where a high level of resolution is required to verify a prediction or make a decision.

Field Verification

116. A primary objective of the FVP was to field verify the laboratory biological responses by measuring the same response under both laboratory and field exposures in the same species. A basic and often implicit assumption is that results derived from laboratory tests are directly applicable in the

field. The null hypothesis, explicitly stated, is that there are no significant differences in the exposure-response relationships measured for the same species in both the laboratory and the field. The acceptance of this hypothesis is necessary to extrapolate laboratory predictions to the field with some degree of confidence. However, a rigorous statistical test of this hypothesis requires defining field exposures at a level of resolution comparable to that in the laboratory. Because our field exposure data are discrete rather than continuous, the comparison between laboratory and field responses was of necessity limited to comparing exposure boundaries rather than discrete absolute values.

117. The results support the hypothesis that, given comparable exposures in the laboratory and field, certain specific biological response patterns can be expected to concur. In *M. edulis*, the laboratory-derived response threshold for SFG was 1.5 mg/l suspended BRH sediment. Based upon both chemical analysis and tissue residue values (Figure 3), the field exposure values were generally at or below 1.5 mg/l during disposal and immediately postdisposal at 1 m above the bottom. The corresponding field values for SFG were not different from the REF values except during the disposal and immediate postdisposal periods when the estimated exposures reached the laboratory-derived response threshold. Thus, laboratory predictions agreed with the field measurements of this parameter. Similar results were observed for bioaccumulation patterns in *M. edulis*, which paralleled laboratory studies in terms of the types of compounds accumulated, the distribution of PCB congeners, and the actual quantitative values for the major contaminants. Field histopathological responses in *M. edulis* were generally in agreement with laboratory responses, the exception being the absence of gill pathology at the highest field exposure. Based upon laboratory data and the duration and intensity of field exposure, one would not predict that histological effects would occur in the field. This was the case since no effects were detected in field-exposed mussels. Adenylate energy charge measured in *M. edulis* did not show an exposure-response relationship in the laboratory and thus would not be expected to respond in the field. Examination of the field data confirmed this prediction. These data for *M. edulis* support the verification of laboratory responses in the field when comparable exposures are operative.

118. The field verification of responses in *N. incisa* followed a similar pattern. The bioenergetics patterns for respiration measured in previous

laboratory exposures and those for excretion in this study predicted effects at mound stations postdisposal, which is what was observed. The only histological response detected in the laboratory was a thickening of the parapodial epidermis. The fact that field-exposed worms did not exhibit this response was probably due to differences in total exposure. Effects on AEC were not detected in laboratory exposures. Statistical differences were detected between stations for one sampling period in the field, but these differences were not biologically significant. The SCE response was inconsistent in replicated laboratory experiments. However, there was an increased frequency in SCE in *N. incisa* exposed to BRH sediments when all the laboratory data were pooled. A similar magnitude of increase in SCE frequency was observed in field-exposed worms. This SCE increase in field-exposed worms declined to the control values when the worms were held in REF sediment in the laboratory. Finally, the contaminant bioaccumulation patterns measured in *N. incisa* in the laboratory and field showed a high degree of concurrence.

119. The objective of the field verification component of the FVP was to verify the responses measured in the laboratory with those observed in the field. The laboratory and field data collected on *M. edulis* and *N. incisa* indicate that the laboratory responses can be used to predict field responses with an acceptable degree of confidence. However, these studies have emphasized the importance of defining the field exposure environment when interpreting biological response data.

Residue-Effects Relationships

120. The relationships between contaminant tissue residues and the biological responses measured in the FVP were examined using correlation analysis, which does not imply cause and effect when dealing with complex mixtures such as sediments. As expected, the biological responses that showed an exposure-response relationship to BRH suspended sediment concentration also showed a similar relationship to contaminants that were bioaccumulated. In general, the PCBs were the most strongly correlated with the biological responses and were closely followed by the higher molecular weight PAHs. Of the metals, only copper and cadmium were correlated with biological responses in *M. edulis*. Chromium was correlated with a biological response in *N. incisa*. These studies demonstrate that there is a relationship between

biological responses and tissue residues of those contaminants that are bio-accumulated and not readily biodegraded. The issue of residue and effects is complex. It involves not only the uptake of parent contaminants but also their metabolism and the effects of their metabolites, which are often more toxic. Interpretation of residue-effects relationships involving complex mixtures such as sediments is further compounded by potential synergistic/antagonistic effects. This study was not designed to address these issues.

PART IV: CONCLUSIONS

121. The Field Verification Program was designed to test the applicability, reproducibility, and verification of biological test methods to be used in the evaluation of dredged material impacts in aquatic, wetland, and upland disposal sites. Comparison of the three disposal options will be discussed in the overall FVP synthesis report. The details and results of the research summarized in this report are presented in a series of published technical reports.

122. The aquatic portion of the FVP is comprised of three components: (a) laboratory documentation, (b) field verification, and (c) residue-effects relationships. The following paragraphs summarize the conclusions derived from the aquatic portion of the FVP and provide recommendations for use of the test methods.

Conclusions

Laboratory documentation

123. The objectives of these studies were to determine if the test methods could quantitatively and predictably describe biological responses to dredged material exposure in a variety of marine species and to determine the variability and reproducibility of the response to replicate exposures of dredged material. The results, summarized in Table 6, are discussed below.

- a. The ability of the biological test methods to respond quantitatively to varying exposures of dredged material was demonstrated for all responses except adenylate energy charge, sister chromatid exchange, and histopathological responses in *N. incisa*.
- b. The reproducibility of the test methods was assessed in the laboratory through replicate tests with dredged material. Only adenylate energy charge, sister chromatid exchange, and histopathology did not replicate in a quantitatively acceptable manner. Histopathological responses were qualitatively reproducible in that similar lesions occurred in the same species repeatedly; however, the response frequency was not reproducible.

124. The laboratory documentation phase of the program demonstrated that selected biological test methods could be used to reproducibly measure the effects of dredged material in the laboratory. Having applied these

Table 6

Success and Applicability of Test Methods for Evaluating Dredged Material Effects

Biological Response	Laboratory Documentation			Field Verification	Residue Effects	Regulatory Application	
	Exposure-Response Relationship	Replicate Reproducibility of Test Method				Predisposal Evaluation	Monitoring Application
Bioaccumulation							
<i>M. edulis</i>	+	+		+	NA	+	+
<i>N. incisa</i>	+	+		+	NA	+	+
Scope for growth							
<i>M. edulis</i>	+	+		+	+	+	+
Bioenergetics							
<i>N. incisa</i>	+	+		-	+	-	-
AEC							
<i>M. edulis</i>	-	-		+	+	-	-
<i>N. incisa</i>	-	-		+	+	-	-
SCE							
<i>N. incisa</i>	-	-		+	+	-	-
Histopathology							
<i>M. edulis</i>	+	+		-	+	-	+
<i>N. incisa</i>	-	-		-	-	-	+
Mortality							
<i>M. bahia</i>	+	+		NA	NA	+	-
<i>A. abdita</i>	+	+		NA	NA	+	+

(Continued)

Note: NA (not applicable), + (successful), - (unsuccessful).

Table 6 (Concluded)

Biological Response	Laboratory Documentation				Field Verification	Residue Effects	Regulatory Application	
	Exposure-Response Relationship	Replicate	Reproducibility of Test Method				Predisposal Evaluation	Monitoring Application
Growth								
<i>M. edulis</i>	+	+	+	+	+	+	+	+
<i>N. incisa</i>	+	+	+	NA	NA	NA	+	-
<i>M. bahia</i>	+	+	+	NA	NA	NA	+	-
<i>A. abdita</i>	+	+	+	NA	NA	NA	+	-
Reproduction								
<i>M. bahia</i>	+	+	+	NA	NA	NA	+	-
<i>A. abdita</i>	+	+	+	NA	NA	NA	+	-
Population Growth								
<i>M. bahia</i>	+	+	+	NA	NA	NA	+	-
<i>A. abdita</i>	+	+	+	NA	NA	NA	+	-
Community Benthic REMOTS	NA	NA	NA	NA	NA	NA	+	+
	NA	NA	NA	NA	NA	NA	+	+

methods in the laboratory, the next phase of the program was to evaluate the test methods in the field and compare the results with laboratory predictions.

Field verification

125. The objective of the field verification component of the program was to verify the laboratory test methods and predictions in the field. Stated as a null hypothesis, the exposure-response relationship developed in the laboratory is not significantly different from a similar relationship in the field. To test this hypothesis it is necessary to describe the field exposure conditions to the same level of detail as is done in the laboratory. Continuous, comprehensive time-variable field exposure information was not acquired in this study. Consequently, field exposures were estimated from discrete data that limit the degree of quantification of the field verification. Therefore, rather than attempt to precisely define the exposure field, upper and lower boundaries were estimated and used to compare with the laboratory exposures. The results (summarized in Table 6) are as follows:

- a. The test methods for measuring bioaccumulation, scope for growth, bioenergetics, adenylate energy charge, sister chromatid exchange, and histopathology were successfully applied to the same species in both the laboratory and field. However, adenylate energy charge and sister chromatid exchange did not respond quantitatively to BRH exposure.
- b. There was excellent laboratory and field concurrence with the bioaccumulation of PCBs and PAHs, their chemical patterns, and the magnitude of accumulation in both *M. edulis* and *N. incisa*.
- c. Scope for growth in *M. edulis* showed concurrence between the laboratory and field when analogous exposure conditions were compared. Significant changes in field scope for growth measurements in *M. edulis* were limited to within 2 months after disposal and reflect the transient nature of the water column exposure. The bioenergetics responses of field populations of *N. incisa* that were exposed via the sediments continued to show statistically significant changes 2 years after disposal. In addition, there was concurrence between laboratory and field measures of respiration and excretion in *N. incisa*.
- d. Adenylate energy charge and related adenylate nucleotides were measured in *M. edulis* and *N. incisa* in both the laboratory and field. These responses, with the exception of the adenine pool, did not show a quantitative exposure-response relationship. The adenylate analyses in field *N. incisa* were complicated by technical problems with the measurement of the adenine nucleotides from field samples during the first 3 months after disposal. Considering these limitations, it is not surprising that attempts to correlate laboratory and field responses were unsuccessful.

- e. Sister chromatid exchanges, measured in *N. incisa*, were determined in both the laboratory and the field. The laboratory response, while present, lacked a predictable exposure-response relationship and did not replicate satisfactorily. An increase in SCE exchanges was detected in field samples collected during one postdisposal sampling period. Thus, the degree of concurrence between laboratory and field is essentially a qualitative one in that an increase in the SCE response was detected in both lab and field in the presence of BRH sediment.
- f. Histopathological effects were measured in *M. edulis* and *N. incisa* in both the laboratory and field. The lack of concurrence between laboratory and field histopathological responses in *M. edulis* was unexpected based on the laboratory data. A possible explanation for this discrepancy is the difference between the total integrated exposure between the laboratory and field. Histopathological effects in *N. incisa* were not detected in the field even at exposures that overlapped those in the laboratory where effects were detected. The laboratory effect, 18-percent incidence, is probably not significant in view of the sample age (N = 11). Thus, the inability to detect effects in the field could be within the variability of the measurement, resulting in a concurrence between laboratory and field.

126. The above summarizes the field verification of selected laboratory responses. It is imperative that comparisons be made for analogous exposures in order to properly interpret the biological data. It is not surprising that those responses that did not perform well under laboratory conditions were the same ones that could not be verified in the field. Based upon the data in this study it is concluded that with analogous exposures, exposure-response relationships developed in the laboratory are not qualitatively or quantitatively different from those in the field.

Residue-effects relationships

127. The purpose of this component of the study was to determine if correlations existed between specific contaminant tissues residues and observed biological responses. Bioaccumulation data provided evidence that contaminants were biologically available. This is of fundamental importance to establishing exposure patterns and supporting those developed from chemical analysis. However, bioaccumulation data alone do not provide evidence of biological consequences or effects upon the organism. The approach taken in this study was correlative in nature and did not seek to demonstrate cause-and-effect relationships from exposure to the complex of contaminants identified

in BRH dredged material. The principal conclusions from these studies are summarized below.

- a. The principal residue-effect relationships were identified in the laboratory for scope for growth and histopathology measured in *M. edulis*, and for bioenergetics in *N. incisa* in the laboratory and in limited cases in the field. Residue-effects relationships were also detected for adenylate energy charge in both *M. edulis* and *N. incisa* in the laboratory, but the biological significance is not important since all the AEC values were within the normal range. Residue-effects relationships were detected for sister chromatid exchange in *N. incisa* in a single set of field samples.
- b. The most consistent correlations were achieved with PCBs and the higher molecular weight PAHs which are refractory to metabolic degradation and detoxification.

Recommendations

128. The following recommendations are presented in terms of the utility of the methods in predisposal evaluation of dredged material impacts and in postdisposal compliance monitoring and assessment studies (Table 6).

- a. Of the test methods applied and evaluated in this program, measures of mortality, scope for growth, reproduction, and population responses are recommended for predisposal permit testing. In addition to *A. abdita* and *M. bahia*, consideration should be given to expanding the suite of test species to represent the range of sensitive organisms expected in the environment.
- b. Measures of bioaccumulation are of fundamental importance in determining the bioavailability and exposure to contaminants in complex materials. Experience suggests that test methods for both bedded and suspended sediment exposures are important. Both *M. edulis* and *N. incisa* performed well in these studies and should be considered in a suite of test species for bioaccumulation.
- c. A genotoxic test method is also recommended for inclusion in the predisposal permit testing program when there is reason to believe that the concentration of genotoxic contaminants is sufficiently great as to be of concern. However, SCE is not adequately developed to meet this need. Although an in-depth analysis of the Ames test was not performed, it is suggested that it, along with other genotoxic methods, be evaluated for predisposal evaluation of sediments.
- d. The development, application, and evaluation of benthic assessment methods suggest that a rapid reconnaissance technique, such as REMOTS, be utilized during predisposal site

evaluation. The results from this technique corresponded with other measures of benthic community structure and health.

- e. Several of the methods evaluated in this program have utility for postdisposal field assessments. These include scope for growth, growth, and bioaccumulation measured in *M. edulis* and bioenergetics and bioaccumulation measured in *N. incisa*. The benthic community assessment methods are both recommended for field assessments. The REMOTS method should be used as a rapid reconnaissance tool to identify and characterize the spatial and temporal extent of impacts and to direct more intensive sampling for detailed species enumeration.
- f. Finally, the implementation manual currently used for evaluating dredged material impacts is in need of revision. When the methods recommended in this manual were applied to BRH dredged material, no toxicity was detected. This contradicts the results reported in this program. Consequently, additional species and biological test methods need to be incorporated into revisions of the manual. These toxicity tests may be of longer duration and more sophisticated than those currently recommended. Measures of bioaccumulation in *N. virens* used in the current implementation manual did concur both qualitatively and quantitatively with the results obtained in this study with *N. incisa*. However, concurrence was not found in comparative tests with *Mercenaria mercenaria* and *M. edulis*.

129. The conclusions from this study demonstrate that a variety of test methods can be applied to the evaluation of dredged material impacts. These methods have been verified under field conditions and should be considered for incorporation into changes in the current regulatory testing requirements.

REFERENCES

- Atkinson, D. E. 1971. "Regulation of Enzyme Function," Annual Review of Microbiology, Vol 23, pp 47-68.
- Atkinson, D. E., and Walton, G. M. 1967. "Adenosine Triphosphate Conservation in Metabolic Regulation; Rat Liver Citrate Cleavage Enzyme," Journal of Biological Chemistry, Vol 242, pp 3239-3241.
- Bickford, T. J. 1975. "The Geochemistry of Chromium in Narragansett Bay, Rhode Island," M.S. thesis, Brown University, Providence, R. I.
- Bloom, S. E. 1978. "Chick Embryos for Detecting Environmental Mutagens," Chemical Mutagens; Principles and Methods for Their Detection, A. Hollaender and F. J. de Serres, eds., Plenum Press, New York, Vol 5, pp 203-232.
- Chapman, A. G., Fall, L., and Atkinson, D. E. 1971. "Adenylate Energy Charge in *Escherichia coli* During Growth and Starvation," Journal of Bacteriology, Vol 108, pp 1072-1086.
- Fries, C. R., and Lee, R. F. 1984. "Pollutant Effects on the Mixed Function Oxygenase (MFO) and Reproductive Systems of the Marine Polychaete *Nereis virens*," Marine Biology, Vol 79, pp 187-193.
- Gardner, G. R., Yevich, P. P., Malcolm, A. R., Rogerson, P. F., Mills, L. J., Senecal, A. G., Lee, T. C., Harshbarger, J. C., and Cameron, J. P. 1986. "Tumor Development in American Oysters and Winter Flounder Exposed to a Contaminated Marine Sediment Under Laboratory and Field Conditions" (Abstract), Symposium on Toxic Chemicals and Aquatic Life, September 16-18, 1986, Seattle, Wash.
- Gentile, J. H., and Scott, K. J. 1986. "The Application of a Hazard Assessment Strategy to Sediment Testing: Issues and Case Study," Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems, K. L. Dickson, A. W. Maki, and W. Brungs, eds. Proceedings of Sixth Pellston Workshop, August 1984, Florissant, Colo., SETAC Special Publication, pp 1-17.
- Gentile, J. H., Scott, K. J., Lussier, S., and Redmond, M. S. 1985. "Application of Laboratory Population Response for Evaluating the Effects of Dredged Material," Technical Report D-85-8, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Gentile, J. H., et al. 1987. "The Assessment of Black Rock Harbor Dredged Material Impacts on Laboratory Population Responses," Technical Report D-87-3, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Ivanovici, A. M. 1980. "Adenylate Energy Charge: An Evaluation of Applicability to Assessment of Pollution Effects and Directions for Future Research," Rapport et Process-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer, Vol 179, pp 23-28.

Johns, D. M., and Gutjahr-Gobell, R. 1988. "Bioenergetic Effects of Black Rock Harbor Dredged Material on the Polychaete *Nephtys incisa*: A Field Verification," Technical Report D-88-3, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Johns, D. M., Gutjahr-Gobell, R., and Schauer, P. 1985. "Use of Bioenergetics to Investigate the Impact of Dredged Material on Benthic Species: A Laboratory Study with Polychaetes and Black Rock Harbor Material," Technical Report D-85-7, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Karickhoff, S. W., and Morris, K. R. 1986. "Pollutant Sorption: Relationship to Bioavailability," Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems, K. L. Dickson, A. W. Maki, and W. Brungs, eds., Proceedings of Sixth Pellston Workshop, August 1984, Florissant, Colo.

Lake, J. L., Hoffman, G. L., and Schimmel, S. E. 1985. "The Bioaccumulation of Contaminants from Black Rock Harbor Dredged Material by Mussels and Polychaetes," Technical Report D-85-2, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Lake, J. L., Rubinstein, N., and Pavignano, S. 1986. "Predicting Bioaccumulation: Development of a Simple Partitioning Model for Use as a Screening Tool for Regulating Ocean Disposal of Wastes," Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems, K. L. Dickson, A. W. Maki, and W. Brungs, eds., Proceedings of Sixth Pellston Workshop, August 1984, Florissant, Colo.

Latt, S. A., Allen, J., Bloom, S. E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., and Wolff, S. 1981. "Sister Chromatid Exchange: A Report of the Gene-Tox Program," Mutation Research, Vol 87, pp 17-62.

Lee, R. F., and Singer, S. C. 1980. "Detoxifying Enzymes System in Marine Polychaetes: Increases in Activity After Exposure to Aromatic Hydrocarbons," Rapport et Process-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer, Vol 179, pp 29-32.

Lee, R. F., Stolzenbach, J., Singer, S., and Tenore, K. R. 1981. "Effects of Crude Oil on Growth and Mixed Function Oxygenase Activity in Polychaetes, *Nereis* sp.," Biological Monitoring of Marine Pollutants, F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg, eds., Academic Press, New York, pp 323-324.

MacKay, D., and Patterson, S. 1981. "Calculating Fugacity," Environmental Science and Technology, Vol 15, pp 1006-1014.

McCall, P. L. 1977. "Community Patterns and Adaptive Strategies of the Infaunal Benthos of Long Island Sound," Journal of Marine Research, Vol 35, pp 221-266.

McFarland, V. A. 1984. "Activity-Based Evaluation of Potential Bioaccumulation from Sediments," Dredging '84 Proceedings, American Society of Civil Engineers, Vol 1, pp 461-467.

McFarland, V. A., and Clarke, J. U. 1986. "Testing Bioavailability of Polychlorinated Biphenyls from Sediments Using a Two-Level Approach," Proceedings, USAE Committee on Water Quality, Sixth Seminar, The Hydrologic Engineering Center, Davis, Calif., pp 220-229.

Michael, A. D. 1975. "Structure and Stability in Three Marine Benthic Communities in Southern New England," E. Monowitz, ed., Brookhaven Symposium on the Effects of Energy Related Activities on the Outer Continental Shelf, pp 109-125.

Mills, E. L. 1967. "The Biology of an Ampeliscid Amphipod Crustacean Sibling Species Pair," Journal of the Fisheries Research Board of Canada, Vol 24, pp 305-355.

Nelson, W. G., Black, D., and Phelps, D. 1985. "Effects of Black Rock Harbor Suspended Sediment on the Scope for Growth of the Blue Mussel, *Mytilus edulis*: A Laboratory Evaluation," Technical Report D-85-6, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Nelson, W. G., et al. 1987. "Effects of Black Rock Harbor Dredged Material on the Scope for Growth of the Blue Mussel, *Mytilus edulis*, After Laboratory and Field Exposures," Technical Report D-87-7, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Perry, P., and Evans, J. H. 1975. "Cytological Detection of Mutagen-Carcinogen Exposure by Sister Chromatid Exchange," Nature (London), Vol 258, pp 121-125.

Pesch, G., Pesch, C. E., and Malcolm, A. R. 1981. "*Neanthes arenaceodentata*, a Cytogenetic Model for Marine Genetic Toxicology," Aquatic Toxicology, Vol 1, pp 301-311.

Pesch, G. G., et al. 1987. "Sister Chromatid Exchange in Marine Polychaetes Exposed to Black Rock Harbor Sediment," Technical Report D-87-5, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Phelps, D. K. 1958. "A Quantitative Study of the Infauna of Narragansett Bay in Relation to Certain Physical and Chemical Aspects of Their Environment," M.S. thesis, University of Rhode Island, Kingston, R. I.

Rhoads, D. C., Aller, R. C., and Goldhaber, M. B. 1977. "The Influence of Colonizing Benthos on Physical Properties and Chemical Diagenesis of the Estuarine Seafloor," B. C. Coull, ed., Ecology of Marine Benthos, University of South Carolina Press, Columbia, S. C., pp 113-138.

Rhoads, D. C., and Germano, J. D. 1982. "Characterization of Organism-Sediment Relations Using Sediment Profile Imaging: An Efficient Method of Remote Ecological Monitoring of the Seafloor (REMOTS System)," Marine Ecology Program Series, Vol 8, pp 115-128.

Rogerson, P. F., Schimmel, S. C., and Hoffman, G. 1985. "Chemical and Biological Characterization of Black Rock Harbor Dredged Material," Technical Report D-85-9, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

- Sanders, H. L. 1956. "Oceanography of Long Island Sound, 1952-1954; X. Biology of Marine Bottom Communities," Bulletin of Bingham Oceanographic Collection, Yale University, Vol 15, pp 345-414.
- _____. 1958. "Benthic Studies in Buzzards Bay; I. Animal-Sediment Relationships," Limnology and Oceanography, Vol 38, pp 265-380.
- Scott, K. J., et al. 1987. "The Impact of Open-Water Disposal of Black Rock Harbor Dredged Material on Benthic Recolonization at the FVP Site," Technical Report D-87-4, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Sinnett, J. C., and Davis, W. R. 1983. "A Programmable Turbidistat for Suspended Particles in Laboratory Aquaria," Journal of Experimental Marine Biology and Ecology, Vol 73, pp 167-174.
- Skjoldal, H. R., and Bakke, T. 1978. "Relationship Between ATP and Energy Charge During Lethal Metabolic Stress of the Marine Isopod *Cirrolana borealis*," Journal of Biological Chemistry, Vol 253, pp 3355-3356.
- Solomon, E., and Bobrow, M. 1975. "Sister Chromatid Exchanges: A Sensitive Assay of Agents Damaging Human Chromosomes," Mutation Research, Vol 30, pp 273-278.
- US Army Corps of Engineers. 1987. "Simplified Approach for Evaluating Bioavailability of Neutral Organic Chemicals in Sediment," Environmental Effects of Dredging Technical Note EEDP-01-8, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- US Environmental Protection Agency/US Army Corps of Engineers. 1977. "The Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters; Implementation Manual for Section 103 of PL 92-532," Environmental Effects Laboratory, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Yevich, P. P., Yevich, C., Pesch, G., and Nelson, W. 1987. "Effects of Black Rock Harbor Dredged Material on the Histopathology of the Blue Mussel *Mytilus edulis* and Polychaete Worm *Nephtys incisa* After Laboratory and Field Exposures," Technical Report D-87-8, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Yevich, P. P., et al. 1986. "Histopathological Effects of Black Rock Harbor Dredged Material on Marine Organisms; A Laboratory Investigation," Technical Report D-86-1, prepared by US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
- Zaroogian, G. E., et al. 1985. "Laboratory Evaluation of Adenylate Energy Charge as a Test for Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material," Technical Report D-85-3, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Zaroogian, G. E., et al. 1988. "A Field and Laboratory Study Using Adenylate Energy Charge as an Indicator of Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material," Technical Report D-88-4, prepared by the US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.